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GUINEA PIG VIRULENCE AND CATALASE ACTIVITY OF ISONIAZID-RESISTANT TUBERCLE BACILLI*

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The Problem

Isoniazid-resistant tubercle bacilli which seem to be avirulent when inoculated into guinea pigs have been a puzzling phenomenon in tuberculosis bacteriology laboratories. These guinea pigs have generally been sacrified at 8 weeks though they have a positive Mantoux test at 4 weeks.

At 8 weeks some of the guinea pigs seem to have minimal lesions of the liver and spleen without acid-fast bacilli. Occasionally granulomata have been observed in microscopic sections. Therefore some guinea pigs were sacrificed at an earlier date (4 to 6 weeks) to see if acute regressive lesions were present. Another guinea pig was sacrificed at 8 weeks as a control.

Catalase activity and isoniazid resistance of the cultures were determined since several authors have reported that isoniazid-resistant tubercle bacilli have diminished catalase activity and little or no guinea pig pathogenicity.

Method

Forty-two isoniazid-resistant cultures were isolated on modified Lowenstein-Jensen medium. They were partially to completely resistant to 1 mcg. isoniazid per ml. These cultures were standardized with Hopkins vaccine centrifuge tubes and 1 mg. of culture in 1 cc. was inoculated into the thigh of each of 2 guinea pigs and 1 tube of modified Lowenstein-Jensen medium. Male guinea pigs of mixed colors weighing approximately 350 grams were used. One guinea pig was sacrificed at 4 weeks; one

* Second Award, Scientific Products Foundation, Bacteriology 1956, read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

guinea pig was sacrificed at 8 weeks. A Mantoux test was performed at 4 weeks.

Gross lesions of the liver, spleen, lung and lymph nodes were noted and recorded as negative to 4 plus. Smears were made by crushing the questionable area between 2 slides. Both slides were examined after Ziehl-Neelsen staining.

Most of the specimens were fixed in formalin and microscopic sections were prepared when positive to doubtful lesions were observed and no acid-fast bacilli were found. No microscopic section was considered positive for tuberculosis unless acid-fast bacilli were found.

All of the 42 cultures inoculated into guinea pigs grew well on modified Lowenstein-Jensen medium and were in a growing phase when inoculated into the guinea pigs. Variations in the susceptibility or resistance of individual guinea pigs were not controlled in this study.

Results

Table I summarizes the guinea pig virulence tests. It is to be noted that 14 cultures were positive at 4 weeks and negative at 8 weeks.

TABLE I

Virulence of 42 Cultures in Guinea Pigs.

	Number	Percent
Guinea pig positive at 4 and 8 weeks	22	53%
Guinea pig positive at 4; negative 8	14	33%
Guinea pig negative at 4; positive 8	3	7%
Guinea pig negative at 4 and 8 weeks	3	7%
	42	100%

Table II summarizes the incidence of acid-fast bacilli in microscopic sections. Nineteen animals showed acid-fast bacilli in sections of lesions which were not seen in smears. Seventeen out of 84 guinea pigs had gross lesions but no acid-fast bacilli in smears or microscopic sections. This phenomenon originally instigated the study.

TABLE II

Acid-Fast Bacilli in Microscopic Sections of 84 Guinea Pigs.

	Number	Percent
Positive gross; positive smears	34	41%
Positive gross; negative smears; positive microscopic	19	23%
Negative gross; negative smears; positive microscopic	3	4%
Doubtful gross; negative smear; positive microscopic	2	2%
Positive to ± gross; negative smears; negative microscopic	17	20%
Negative gross; negative smear; negative microscopic	7	8%
Negative gross; positive smears	2	2%
	84	100%

An isoniazid-resistant culture was inoculated into 3 CF_1 male mice (0.2 mg. intravenous). The mice were killed after 21 days and were found to be tuberculous (Table III). This culture produced tuberculosis in guinea pigs at 4 weeks but negative results at 8 weeks.

TABLE III

INH-Resistant Tubercle Bacilli in Mice.

GROSS LESIONS			LUNG		Spleen	Liver
Liver	Spleen	Lung	H & E	Z-N	H & E	H & E
0	Splenomegaly	? granulomas	3+ granulomas with necrosis	Numerous AFB	Granulomas	3+ portal round cells, 2+ focal necrosis, 4+ granulomas

Catalase activity, as determined by Middlebrook's method, was generally slight to absent. The determinations were repeated several times with the same results (Table IV). All guinea pigs which were grossly negative and smear negative at 4 and 8 weeks had been inoculated with cultures which had

TABLE IV

Catalase Activity of 42 Strains of Isoniazid-Resistant Tubercle Bacilli.

	Number	Percent
Catalase 3+	10	24%
Catalase 2+	3	7%
Catalase 1+	18	43%
Catalase Negative	11	26%
	42	100%

no catalase activity (Table V). However, six catalase-positive cultures which seemed avirulent at 8 weeks produced some evidence of tuberculosis in guinea pigs at 4 weeks. At 4 weeks the catalase-negative cultures were less virulent and at 8 weeks there was tuberculosis with those cultures with zero to 1+ catalase activity.

TABLE V

Catalase Activity and Guinea Pig Virulence of 42 Strains of Isoniazid-Resistant Tubercle Bacilli.

	GUINEA PIG VIRULENCE					
	Positive		Doubtful		Negative	
Weeks	4	8	4	8	4	8
Catalase 3+	9	6	1	3	..	1
Catalase 2+	3	2	..	1	..	..
Catalase 1+	17	11	1	2	..	5
Catalase negative	7	5	..	1	4	5
	36	24	2	7	4	11

Discussion

Isoniazid-resistant strains often exhibited a diminished virulence in guinea pigs. At 8 weeks there were many animals which had gross lesions but negative smears and microscopic sections for acid-fast bacilli. It would seem that these strains are not avirulent. Instead, the early lesions tend to regress and acid-fast bacilli are rare. Further studies are planned to include cultures of all lesions in guinea pigs in order to study the fate of the isoniazid-resistant tubercle bacilli.

Conclusion

If the tubercle bacilli are isoniazid-resistant, it is advantageous to sacrifice inoculated guinea pigs at 4 weeks as well as at 8 weeks because the tuberculous lesions may quickly regress. If facilities are available, it is better to inoculate 2 guinea pigs per culture. Microscopic sections with Ziehl-Neelsen stain may be needed to interpret doubtful lesions in guinea pigs. Even if a guinea pig is grossly negative and smear negative, a few small lesions may be found microscopically.

One should not report *Mycobacterium tuberculosis* as avirulent in the guinea pig unless more than one animal has been inoculated and sacrificed in less than 6 weeks with microscopic sections of the organs.

Mouse inoculations may be advantageous with cultures which are low in virulence for guinea pigs.

Catalase activity is usually absent in isoniazid-resistant strains which are avirulent in guinea pigs. Catalase activity of isoniazid-resistant tubercle bacilli is often slight.

Acknowledgment

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AN ANNOUNCEMENT

The *A.S.M.T. By-Laws* require that the officers whose terms of office expire in June, 1957, be listed in this issue of the *American Journal of Medical Technology.*

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THE ROLE OF CALCIUM SALT IN THE KOLMER COMPLEMENT FIXATION TEST FOR SYPHILIS*

II. Further Studies on the Use of Calcium Chloride as an Adjunct to Higher Titer Complement

ELEANOR STACKHOUS, M.T. (ASCP)
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Previous writers have suggested the use of 0.04% calcium chloride as an additive to Kolmer salt to enhance the activity of complement in the Kolmer complement fixation test for syphilis.[1,2,3] Dr. Kolmer has stipulated that, even though the complement was of higher titer than 1:43 in the preliminary titration, it should never be used in a higher dilution than 1:43 in the tests. The reason for this was not given. It seems worthwhile to examine this phase and evaluate the results.

All blood specimens submitted for routine serologic tests for syphilis in a general hospital were run in duplicate, using Kolmer salt and Kolmer salt plus .04% calcium chloride. Twenty-five preliminary titrations were run. The Hemolysin titers ranged from 2 units of 1:1000 to 1:6000 with both Kolmer salt and Kolmer salt plus calcium salt. The complement titers with Kolmer salt ranged from 2 full units of 1:27 to 1:43, while those with calcium salt ranged from 1:37 to 1:60. The one time the titers with Kolmer salt were 1:2000 and 1:27, the corresponding titers with calcium salt were 1:2500 and 1:50. The one time the complement titer with calcium salt was as low as 1:37, the Hemolysin titer was 1:4000 and the Kolmer salt gave the same results.

TABLE 1

25 Complement Titrations

No. of Titrations	Kolmer NaCl	No. of Titrations	Kolmer NaCl Plus $CaCl_2$
1	1:27		
3	1:30		
10	1:33		
9	1:37	1	1:37
2	1:43	7	1:43
		14	1:50
		3	1:60

In 24 titrations the titer of complement was 1 to 5 tubes higher[3] with the calcium salt than with the Kolmer salt alone. There were 17 titrations in which the titer was 1:50 or 1:60 with calcium salt. On the basis of their respective titers for Hemolysin and complement, simplified Kolmer 2 tube tests were run on each serum in duplicate. Simultaneously VDRL tests were also run.

All readings were made for their maximum reactivity based

* Second Award, Scientific Products Foundation, Serology, 1956. Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

on an average secondary water bath incubation of 20-25 minutes. No native amboceptor was absorbed. In about one half the runs, the tests with calcium chloride took 5-10 minutes longer to clear than those with Kolmer salt alone. Any slight inhibition as ± or 1 + in either or both tests was read which increase the reactive results.

This evaluation is based on a total number of 2000 sera and 25 spinal fluids. The results were as follows:

TABLE 2

Complete Agreement (all three tests)			Disagreement	% Disagreement
Non-reactive	1831 sera	24 spinal fluids		
Reactive	36 sera	0 spinal fluids		
			133 sera	6.65%
			1 spinal fluid	4.0%

36 sera were reactive in all 3 tests. This is 1.3% of the 2000 sera tested.
There were 169 sera showing some reactivity in one or more of the three tests.

TABLE 3

Analysis of 169 Reactive Sera

	Non-Reactive	Anti-C	WEAKLY REACTIVE		Reactive	% Weakly Reactive and Reactive
			Plus Minus 1 Plus	2 Plus	3 or 4 Plus	
$CaCl_2$	7	11	58	9	84	89
Kolmer	72	4	40	6	47	55
VDRL	122	..	22		25	28

In Table 3, there are tabulated a number of sera which were read as a plus minus or one plus. On the basis of a single test these would have been reported as non-reactive. Calculated on this basis, the reactive sera with calcium chloride would be reduced to 93 or 55%. With Kolmer salt the reactive sera would have been 53 or 31%. On the basis of 2000 sera tested, the 93 reactive sera with calcium chloride would be 4.15% reactive and 53 reactive sera in Kolmer salt alone would be 2.65% reactive.

TABLE 4

Reactions with Kolmer NaCl and Kolmer NaCl plus $CaCl_2$

AGREEMENT					Disagreement Total
Non-Reactive	Anti-C	Weakly Reactive	Reactive	Total	
3	4	21	44	72 or 43.0%	97 or 57%

For the years 1953, 1954 and 1955 at this hospital the percentage of reactive sera using the Kolmer and VDRL tests was 4.1, 3.9, 3.0 respectively. In the present series of 2000 sera, there were 55 or 2.75% reactive using the Kolmer and VDRL tests.

There was no attempt to correlate these reactions with the clinical findings or case history. However, there were 36 sera

from patients from the syphilis clinic, 24 of which were among the 36 sera reacting to all three tests.

Summary

1. 2000 sera were tested by performing the Kolmer simplified two tube test for syphilis using Kolmer salt, Kolmer salt plus .04% calcium chloride and the VDRL flocculation test. The titer of the Complement with calcium chloride was 1:43 or higher in all but one instance. (Table 1.) It was used at its 2 full unit titer.

2. Results showed:

1831 sera non reactive
36 sera reactive to all three tests
93 sera reactive with $CaCl_2$ alone
53 sera reactive with Kolmer salt alone
47 sera reactive with VDRL alone
133 variable reactions in all three tests
97 disagreements between the Kolmer and $CaCl_2$ salt
169 sera reacting to one or more of the 3 tests

3. With the calcium chloride salt there were twice as many reactive sera as with the VDRL.

4. There were 11 Anti-Complementary reactions with $CaCl_2$, and 4 with Kolmer. This is almost a three fold increase with $CaCl_2$.

Conclusion

1. The addition of calcium chloride to Kolmer salt increased the activity of the complement.

2. It also increased the reactivity of the complement fixation test to the point of being more sensitive or non specific as compared with other tests.

3. It increased the anti-complementary reactions.

4. The larger the battery of tests used the more discrepant the results.

5. The use of complement in a dose higher than 1:43 did not seem to be too little complement as all controls hemolyzed promptly. The lag in reading time was no more evident in this experiment than had been shown when an arbitrary dose of 1:43 was used.[3]

6. The 97 disagreement between the Kolmer and the calcium chloride was 4.85% of the sera tested which gives evidence that the addition of calcium chloride is not desirable.

7. For the above reasons it would not seem advisable to add calcium chloride to the Kolmer salt when doing the complement fixation tests for syphilis.

(Acknowledgment is made to Sharpe and Dohme for their free contribution of all complement used in the tests, also to Dr. John W. Eiman for his helpful criticism of the paper.)

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UNIDENTIFIED STRAINS OF MYCOBACTERIA ASSOCIATED WITH HUMAN DISEASE*

ALICE TIMPE, M.T. (ASCP)**
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Since the use of chemotherapy, acid-fast bacteria other than the known pathogenic types of mycobacteria are being found with increasing frequency. Many laboratories attach little significance to this group and report them as saprophytes or disregard them entirely. However, a number of cases have been reported where chromogenic acid-fast bacteria were isolated from the patients with pulmonary disease in the absence of typical tubercle bacilli[1-5] and from resected pulmonary tuberculous tissue.[6-8] These organisms were non-pathogenic for guinea pigs. Besides these unidentified chromogenic strains associated with disease, isoniazid resistant strains which are non-pathogenic for guinea pigs have been isolated from patients under therapy.[9] Strains non-pathogenic for guinea pigs and culturally indistinguishable from tubercle bacilli have been isolated from patients having no history of chemotherapy, and have been considered non-pathogenic mycobacteria.[10]

The finding of these strains whose cultural and/or animal virulence studies do not conform to the pattern of typical tubercle bacilli presents a problem in the diagnostic laboratory. If these bacteria cannot be classified as one of the known types of mycobacteria, how should they be reported? Undoubtedly many are non-pathogenic for man, yet others appear to be causing disease.

During a two year period, a series of unclassified strains of mycobacteria isolated from known or suspected tuberculous patients was studied.* The purpose of this study was to attempt to determine etiological relationship of these bacteria to human disease and to establish a means of distinguishing types.

Most of these strains were isolated from sputa or gastric washings. Others came from bronchial fluid, urine, and pleural fluid, while nine were taken from lung tissue obtained at surgery or at autopsy. (Table 1) Strains reported by Buhler & Pallock,[6] Hall,[7] and Richardson[8] were included in this study. A preliminary report of this work was published in 1954.[11] Since publication of the preliminary data, additional cultures have been added to the group. In all, one hundred and nine strains were studied; fifty-two were tested for animal virulence. Known types of mycobacteria: human (H37Rv), bovine, avian, vole, phlei, butyricum, and smegmatis were used for comparison. Colonial

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TABLE I
Sources of Strains

	Sputum	Gastric Washings	Pleural Fluid	Urine	Lymph Node	Bronchial Fluid	Lung Tissue	Water	Unknown
Photo-chromogens.....	12	1	2	..	..	1	7	..	3
Skoto-chromogens.....	25	23	1	2	..	..	..	1	9
Non-photo-chromogens..	17	1	..	..	1	..	2	..	2

and morphological studies were made on 2-3 week old cultures grown on Lowenstein-Jensen medium.[12] Smears of growth were stained by Ziehl-Neelsen technique and examined microscopically. For rate of growth and pigment production transfers were made on Lowenstein-Jensen medium and incubated at 37° C. and 25° C.

In addition, *in vitro* tests which have been reported as a rapid means of distinguishing virulent and non-virulent tubercle bacilli were used. The formation of "serpentine cords" and a relationship with virulent tubercle bacilli has been reported by Middlebrook and workers.[13-44] Correlation between the neutral red test and animal pathogenicity was first reported by Dubos & Middlebrook.[15] Later studies have shown agreement between animal pathogenicity and the neutral red reaction and its usefulness as a rapid *in vitro* test for virulence.[16] It was thought that these tests might aid in classifying the unidentified acid-fast organisms. For the formation of "serpentine cords" cultures were grown in Dubos' liquid Tween albumin medium[12] containing 0.02% Tween and examined after two weeks incubation. Further studies were made with the addition of 5% unheated serum to the Tween albumin medium. To permit a better differentiation of growth pattern, Yegian and Kurung added plasma or serum to the medium and report that this addition enhanced cord formation.[17] For the neutral red test cultures 2 to 3 weeks old were grown on Lowenstein-Jensen medium. Dubos and Middlebrook describe the techniques used.[15]

In vivo studies were made using guinea pigs, dba mice, rabbits, and chickens. Inocula were prepared from 8 to 10 day old cultures grown in Dubos' Tween albumin medium, and standardized by the Hopkins tube method.[12] The animals were injected as follows: guinea pigs—subcutaneous injection with 1 mgm; dba mice—intravenous injection with 0.2 mgm; rabbits and chickens—intravenous injection with 0.01 mgm. Four and six weeks after inoculation tuberculin tests were done on the guinea pigs, rabbits and chickens, using 0.1 ml Old Tuberculin and avian tuberculin diluted 1-10 as the test inoculum.

The surviving animals were sacrificed as follows: guinea pigs

at 8 weeks; dba mice at 8 to 10 weeks; rabbits and chickens at 3 months. The animals were then autopsied and gross microscopic and cultural studies of the organs done.

RESULTS

All the strains studied were strongly acid-fast bacilli. Their staining reaction did not differ from mammalian tubercle bacilli. Variations in morphology ranged from long barred bacilli to coccibacilli; many strains were pleomorphic with one strain showing branching. Nearly all of the strains resembled tubercle bacilli. While differences existed, they were not constant enough to be used as a differentiating criteria in distinguishing them from the tubercle bacilli. Variation in the size and appearance of mammalian tubercle bacilli occurs, depending on such factors as the age of the culture, media used, etc.

All the strains dispersed readily in saline, with the exception of those presenting a rough colonial appearance. The avian, bovine and vole type of tubercle bacilli as well as the known saprophytes also disperse readily in saline, with the exception M. phlei.

The appearance of the colonies on Lowenstein-Jensen media will vary from smooth glistening with entire edge to rough tubercle bacilli like colonies. Some of strains had both smooth and slightly rough colonies; others had smooth centers with slightly irregular thin margins. Originally, these organisms were grouped according to their characteristics as colonies and as to their pigment production. At present, however, grouping is on the basis of pigment production.[18] It may well be that after further study the present grouping criteria may be changed.

Using the current criteria of pigment production these organisms were classified into three groups; photochromogens, skotochromogens and non-chromogens. The first group, photochromogens, appear as buff or cream colored colonies which turn yellow on exposure to light. This pigment occurs only with continued growth. Most of these strains are smooth and glistening colonies with an entire edge. However, there were several rough strains that resembled human tubercle bacilli except in their development of yellow pigment on exposure to light.

The second group, skotochromogens, appear as smooth, glistening, yellow colonies with entire edge when grown in the dark. On exposure to light some strains of these yellow pigmented colonies darken further and may become orange in color.

The non-chromogens which make up the third group are non-pigmented or only slightly pigmented in appearance. They appear as buff or cream colored colonies and do not exhibit any further pigmentation or one so slight as to be negligible. With few exceptions members of this third group of non-chromogens

are smooth with entire edge. Many of them could not be distinguished from avian or vole tubercle bacilli on the basis of pigmentation and colonial type.

On primary isolation, growth of most of these strains appeared after a 2 to 4 week incubation period, but a few took 4 to 8 weeks. On sub-culturing, growth was more rapid. Pin-point colonies could be detected at the end of one week, but maximum growth occurred after 2 to 3 weeks. At 25° C, growth was slower and in most instances the colonies remained very small. With the exception of several strains belonging to the non-chromogenic and skotochromogenic groups, maximum growth was never attained at this temperature even after 6 to 8 weeks. Three strains of the photochromogenic group which were similar to typical tubercle bacilli did not grow at 25° C. The skotochromogens grew better at 25° C than strains belonging to the other groups. For all groups, there was no difference in pigment production when grown at 25° C and 37° C and no growth occurred at 47° C.

When compared with tests on known types of mycobacteria, the rate of growth for these unidentified strains does not differ significantly from that of typical tubercle bacilli. With the exception of a few strains, growth was slower than that of the known saprophytes. Avian and vole bacilli grew as pin-point colonies at 25° C; in this respect many of the unknown strains were similar.

None of the strains tested produced "serpentine cord" comparable to those of typical tubercle bacilli. Four strains belonging to the photochromogenic group whose colonial characteristics were similar to tubercle bacilli formed loose cords. While cord formation was enhanced by the addition of plasma, strains that did not form cords in the medium without plasma remained negative. Comparing these results with those obtained with the known types, H37Rv and M. phlei produced 4+ cords while avian and vole tubercle bacilli and the saprophytes, smegmatis and butyricum were negative (Table II).

Results of the neutral red test done on strains representing all of the three groups of organisms were variable. Some of each group gave a positive reaction. Of the photochromogens, 7/13 gave a positive reaction; 7/11 of the skotochromogens and 4/5 of the non-chromogens tested were positive (Table II). Several workers have reported strains non-pathogenic for guinea pigs and neutral red positive. Morse[16] found 2/168 strains culturally consistent with M. tuberculosis which were neutral red positive and produced no disease in guinea pigs. Twelve chromogens tested gave a negative result. Richmond & Cummings[19] reported one saprophyte isolated from a radish which gave a positive neutral red test. All their strains which were pathogenic for guinea pigs gave a positive cytochemical reaction.

TABLE II

Comparism in Vitro and in Vivo Characteristics

	IN VITRO				IN VIVO			
	Dispersion in Saline	Growth at 25 C.	Neutral Red Test	Serpentine Cords	Guinea Pigs	Mice	Chickens	Rabbits
Photo-chromogens	21 / 25	22* / 25	7 / 13	4 / 25	21 / 21	21 / 21	3 / 3	3 / 3
Skoto-chromogens	61 / 61	50* / 50	7 / 11	47 / 47	15 / 15	14 / 14	1 / 1	1 / 1
Non-chromogens	21 / 23	20* / 20	4 / 5	17 / 17	16 / 16	11 / 15	6 / 6	7 / 7
Tubercle bacilli human type	—	—	+	+	+	+	—	—
Tubercle bacilli avian type	+	+*	+	—	—	+	+	+
Tubercle bacilli vole type	+	+*	+	—	—	+	—	—
M. phlei	—	+	—	+	—	—	—	—
M. smegmatis	+	+	—	—	—	—	—	—
M. butyricum	—	+	—	—	—	—	—	—

* Maximum growth never attained.

None of the unidentified strains tested showed evidence of pathogenicity for guinea pigs. By comparison, 0.1 mgm inoculum of H37Rv produced progressive disease and usually death within 6 weeks. O. T. and avian tuberculin tests gave either no reaction or a weakly positive reaction. Slightly stronger tuberculin reactions were obtained with avian tuberculin but none were as strong as those obtained with H37Rv and avian infected guinea pigs tested with homologous tuberculin. On the basis of the guinea pig as a test animal for pathogenicity of these organisms for man, all of these organisms would be put in the non-pathogenic group.

However, differences in animal pathogenicity could be shown by using mice as test animals. Pulmonary disease in mice, which was sometimes fatal, was produced by all of the photochromogens tested. Lesions could be demonstrated grossly. None of the skotochromogens produced any evidence of disease either by gross or microscopic examination of the tissues. Of the non-chromogens 11/15 produced lesions similar to those of the photochromogenic group. Tubercle bacilli of the human, vole and avian type produced pulmonary lesions similar to those caused by the photochromogenic and non-chromogenic groups whereas the known saprophytes showed no evidence of pathogenicity. No lesions could be demonstrated in rabbits and chickens inoculated with organisms of the three groups (Table II).

The photochromic group and some of the non-chromogens showing pathogenicity for mice have been more closely associated with pulmonary disease than the skotochromogens. They have been isolated from sputum, pleural fluid, and lung tissue in the absence of typical tubercle bacilli, and many have been found repeatedly from the same patient. Direct smears of the pathological material from many patients showed acid-fast bacilli. The source of the skotochromogens and the negative mouse virulence indicates that they may be less significant than the other groups, but they should not be disregarded entirely. Several strains of skotochromogens have been isolated repeatedly from the same patient with pulmonary symptoms, but definite association with disease is lacking. Some have been isolated from patients who have had cultures positive for tubercle bacilli while others have come from patients with no clinical evidence of disease.[8] Repeat cultures were seldom positive for the chromogenic organism; smears of the material cultured were rarely positive. Many of the strains were isolated from gastric specimens and one strain was found in water, indicating possibly that some strains may have been ingested through contaminated water or food.

Some strains may be variants of tubercle bacilli resulting from chemotherapy as these unidentified strains have been found more frequently since the use of antibiotics. However, many of the patients in this study had not been on therapy prior to the finding of these strains. Except for a few patients where cultures were positive for the skotochromogen and also tubercle bacilli, no other pathogenic organisms have been isolated. But the possibility of the strains being secondary invaders is difficult to exclude. It is possible that a virus or bacterium unable to grow on ordinary cultural medium may be the primary pathogen and these strains, non-pathogenic for guinea pigs, may be secondary invaders.

When an acid-fast bacilli whose cultural characteristics do not conform to typical tubercle bacilli is isolated, one must not exclude environmental factors as being responsible for cultural variation. Such things as cultural medium used, moisture, temperature of incubation, age of the culture, may affect the colonial pattern. If the culture medium is moist, tubercle bacilli may appear as smooth colonies not unlike some of the strains discussed in this paper. They will be virulent for guinea pigs and on transfer to a suitable cultural medium will show the typical growth pattern of tubercle bacilli.

Until more evidence is available, the question of pathogenicity of these unidentified strains for man remains unanswered. The source of the strains and the mouse pathogenicity studies strongly indicate that many are causative agents of disease. It is entirely possible that an organism may be pathogenic for man

and non-pathogenic for laboratory animals. One should not rely entirely on laboratory animals to show host-parasite relationships. Further studies should be made on all acid-fast organisms whose cultural and pathogenic pattern does not conform to typical tubercle bacilli or to the known saprophytes.

SUMMARY

Cultural studies were made on 109 strains of unidentified mycobacteria. Of these 52 were studied for animal pathogenicity. All the strains tested were non-pathogenic for guinea pigs, rabbits, and chickens.

None of these mycobacteria other than a few rough strains, produced "serpentine cords." The rate of growth at 37° C does not differ from tubercle bacilli and is slower than the known saprophytes. Best growth occurred at 37° C. Some of the strains gave a positive neutral red test.

The photochromogens were pathogenic for mice and appeared to be closely associated with human disease. Some have been isolated from resected lung tissue.

Strains in the skotochromogens were non-pathogenic for mice. Some have been isolated from patients who also harbor typical tubercle bacilli. In most instances, these are thought to be non-pathogens.

The non-chromogens contain strains virulent and avirulent for mice. Some of these have been associated with human disease. Culturally, many could not be distinguished from avian or vole bacilli.

None of the strains studied conformed to the cultural or pathogenic pattern of typical virulent tubercle bacilli.

In order to shed more light on the nature of these organisms, it is important that laboratories recognize the occurrence of these strains. Further studies should be made of those that appear to be associated with human disease.

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A COLLECTION OF MICRO AND ULTRA-MICRO TECHNIQUES ADAPTED FOR THE GENERAL LABORATORY AND THE BECKMAN SPECTROPHOTOMETER MODEL DU.*

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This collection of micro and ultra-micro techniques is composed of some original work done in our laboratory and methods chosen from various journals, tried and performed to our satisfaction and adapted to our equipment.

The field of micro and ultra-micro techniques is no longer confined to the research laboratory. Its use in the modern laboratory is mandatory in aiding diagnostic and daily examinations on infants and patients whose veins are only available for intravenous feeding and transfusions. In visiting various hospitals this past year it has become increasingly apparent to me that there are not many institutions willing to put the time or money into this new field. There are many reasons for this, the most important being the lack of technologists. However, if the proper evaluation is considered, the accuracy, actual time saved, and undue discomfort to the patient are all factors which add up to the necessity of making time to set up these techniques in the general hospital laboratory. Actually, cost can be kept at a minimum. Most laboratories, now, are finding a flame photometer a necessity. Of course, there are many kinds available and all have salable points. I have used many types and have found the Beckman Model DU very satisfactory because of its wide range of use as a spectrophotometer and flame photometer. Its adaptability to many elements is advantageous and in our laboratory we have had no difficulty in setting up procedures for Na, K, Ca and Mg with great accuracy. The sensitivity of the machine has enabled us to lower our percentage of error to an almost neglible degree.

At the time we set up these techniques our only other large purchase was a pH meter. Its uses in our laboratory not only in Chemistry, but in Histology and Bacteriology made it more useful and economical for us than the purchase of a microgasometer which we hope to invest in in the future. The pH meter is of notable use in checking the blood pH of the premature infant as an aid and guide toward weight gain. The ability to be able to ascertain whether or not acidosis or alkalosis is present is invaluable.

Most all of our glass equipment, such as tubes to draw blood samples and tubes for actual tests, was easily made in the

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** Work done in Chemistry Dept., McLaren Hospital, Flint, Michigan, 1954.

laboratory. Calibrated pipettes are important and these were bought. We found a means of adapting our regular centrifuge to the use of these tubes so the necessity of buying a micro-centrifuge was avoided at this time. Micro-cell cuvets are essential for our spectrophotometer for any test whose final dilution is 3 ml. or less. These are somewhat costly. Any hospital over 100 beds should be equipped to perform any of the following tests discussed in this article. It is suggested that the micro and macro chemistries be divided within the limits of each laboratory. The care of the glassware in itself is important due to expense and the effort of making the various pieces. The solutions are different in most cases and the consistent use of the analytical balance requires more care in making them. Micro and ultra-micro techniques necessitate great patience; accuracy cannot be stressed too much. It is rewarding to note in most instances that these tests performed with skill can produce a more accurate result than most macro methods.

The first thing to consider is: How are we to obtain a blood sample?

A tube of ordinary pyrex having a bore of 1.5 mm. is preferred for the actual collecting of the blood sample. In the event that plasma is desired we use heparinized capillary tubing (0.4% aq. sol. Heparin) for hematocrits and lithium oxylate (1% aq. sol.) for all other chemistries using plasma. Before drawing the blood the oxylate is sucked into the tube and expelled several times leaving a small amount of oxylate fluid in the tubing. For puncture we prefer a Bard-Parker blade. Massage the area to be used for a few seconds and then after wiping with alcohol soaked cotton, puncture the skin. Wipe dry the area of skin and with a gentle drawing, massage the heel, finger or ear lobe toward the wound. Do not squeeze. About a minute is needed to obtain a fairly good flow of blood. Place the tube horizontal to the wound and draw up the blood in a continuous stream. Do not allow air bubbles to enter the tube. Fill the tube from the oxalate end and invert four or five times with a quick snap of the wrist. This will insure uniformity and a clotless sample. To obtain serum, the blood is drawn and set aside to clot. Before spinning, the clot is gently rimmed by a fine wire. Place the tubes in a horizontal rack on a tray so that there is no danger of having them tilt or drop thus losing the sample. Back in the laboratory we use sealing wax in a small evaporating dish. This is heated to melting temperature on a hot plate. Holding the capillary tube horizontally, take a firm hold on the evaporating dish containing the melted wax with the other hand and remove from the hot plate. Tilt the dish sufficiently so that by sliding the capillary tube along the bottom and up the side (still holding the tube horizontally) some of the wax will go up into the

capillary tube. Twist the tube and quickly mold the wax on the outside of the tube into a plug and lay aside to cool for a minute or so.

By placing the capillary tubes into small 3 ml. tubes they may be spun in a regular centrifuge for 10 minutes at 2,000 RPM's. In breaking the spun capillary tube mark the tube with a file just above the column of red cells and snap gently putting pressure on the tube opposite the filed line. It is best to do this with the tube on the bench. The serum can then be expelled into another 3 ml. tube and the desired chemical tests performed.

We have tried, unsuccessfully, various methods of sealing these tubes to insure against leakage. Hemolysis or loss of the sample has occurred and we found that our technicians as a group had better success with the described procedure.

A word of caution concerning the cleanliness of all glassware is important. Glassware for Na and K procedures in our laboratory is washed well, soaked in a cleaning solution of HCL and rinsed twice in distilled water before being dried in the drying oven. All pipettes are treated with acid cleaner and rinsed in a similar manner. Extra care in pipetting is needed and rinsing the pipettes in the solution to be pipetted is advisable. All excess liquid is removed from the outside of the pipette and then the liquid lowered to the appropriate mark. Blow out pipettes are not used in any ultra-micro or micro procedures.

Ultra-Micro Technique Procedures

First I shall deal with the ultra-micro techniques we have employed. *MICRO CHLORIDE*: This can be done on either plasma or serum. If it is possible to do this test in duplicate it is preferable. It can be done on as little as 0.005 ml., but if so the proper aliquots of water and HNO_3 must be determined. The described procedure is on 0.04 ml.

1. Place 0.3 c.c of distilled water in an *evaporating dish*.
2. Place 0.04 c.c serum or plasma in the dish placing the tip of the pipette under the water surface.
3. Place 0.4 c.c of 0.1 N HNO_3 in evaporating dish.
4. Place 4 drops of phenylcarbazone indicator in the dish.
5. Titrate with 0.005 N HNO_3 by introducing the tip of pipette into the liquid and shaking rapidly after adding small increments of acid. Use 0.2 c.c pipette.
6. The end-point is the very first color change and is only slightly pink. Doing this makes a blank unnecessary, but if one prefers to titrate to a definite pink a blank must be run exactly as the unknown with the substitution of water for serum and the titration result subtracted from the unknown and from the standard if a standard is run. Solutions:

To standardize Mercuric Nitrate:

1. Make 0.005 N Mercuric Nitrate: dissolve 0.883 gm. $(HgNO_3)_2$

in 100 ml. of distilled water containing 3 ml. concentrate HNO_3 and make up to 1 liter.

2. Sodium Chloride 0.1N: dissolve 5.845 gm. NaCl (dried at 100°C) and add 3 ml. concentrated HNO_3. Make up to 1 liter with distilled water. Keep bottle well stoppered to avoid evaporation. Dilute 1:10 with 0.3% nitric acid to obtain 0.01 N NaCl.

3. Indicator: s-phenylcarbazone (0.1%: dissolve 100 mg. and make up to 100 c.c with 95% alcohol. Keep in refrigerator and discard when it becomes cherry red.

4. Place 0.1 c.c of 0.01 N NaCl in an evaporating dish, 0.1 c.c of distilled water, 2 drops of indicator and 0.1 c.c of HNO_3 0.1N. It should take 0.2 c.c of $Hg(NO_3)_2$. If so the normality of the mercuric nitrate is exactly 0.005 N and the NaCl is exactly two times as strong. If not calculate the factor.

Calculations: for 0.4 c.c of serum: $\frac{\text{titration of unknown}}{\text{titration of Std.}} \times 100 \div 4 =$ meq. Cl^- expressed as NaCl. To convert meq. Cl to mg% use the following formula: meq. $\div$ 10 $\times$ 58 $\div$ valence = mg%.

If 0.01 or 0.02 c.c of serum is used make the proper substitution in the calculations.

MICRO PROTEIN: As in all micro procedures extreme caution in pipetting is emphasized, such as wiping all excess liquid from pipettes. Take four graduated centrifuge tubes and number them 1 through 4. It is essential to run two standards. The standard should never read higher than 0.008 nor lower than 0.0065 in density. By running two standards the necessity of having to rerun the test is avoided.

1. Into tubes 1, 2 and 3 place 1.0 c.c of distilled water. Into tube 4 which is the blank place 2.0 c.c.

2. Into tube 1, which is the unknown, place 0.02 c.c of plasma or serum.

3. Into tubes 2 and 3, place 0.02 c.c of protein standard (in each).

4. Into all tubes place 2.0 c.c of biuret reagent and place in a 37°C water bath for ten minutes.

5. At the end of ten minutes make up tubes 1, 2, 3 and 4 to 4.0 c.c with distilled water. Invert and read. On the Beckman the settings are: blue phototube, wavelength 550, sensitivity completely clockwise. Selector switch 0.1, slit 0.54.

Solutions: Protein Standard factor: 360 mg./100 c.c or 0.360 gm./100 c.c. Hence .000072 gm. of protein is in 0.02 c.c of standard. The dilution factor is 200.

$$\frac{200}{4} \times .000072 = .36\% \text{ protein in Standard}$$

Calculation: $\frac{RU}{RS} \times .36 =$ gms. % protein.

Biuret Reagent: 1.2 gm. of $CuSO4.5H_2O$ and make up to 100 ml. of water, distilled. 2.5 gm. of NaOH and make up to 100 ml. of water, distilled.

On day of test mix 1:5.

MICRO SODIUM AND POTASSIUM: The standards used in the ultra-micro analysis are identical with those used in the macro procedures. A stock solution of potassium chloride is made consisting of 1.852 gm. of KCl/L which is 25 meq. K/L. A stock solution of Sodium Chloride is also prepared consisting of 2.921 gm. of NaCl/L which has 50 meq. Na/L. The following working standards are made in a pooled fashion so that each standard contains 0.05, 0.10, 015, 0.20, 0.25, 0.3, 0.4, 0.5, and 0.6 micro equiv. of Na and K/cc. The amounts are determined by the following formula:

$0.1(100) : 50 \times (X)$ $\quad X = 0.2$ cc. of Na.
$0.1(100) : 25 \times (X)$ $\quad X = 0.4$ cc. of K.

Thus the second standard would have 0.2 cc. of Na and 0.4 cc. of K made up to 100 cc. with distilled water.

In the macro techniques deproteinization is done with trichloracetic acid. However, there is so little serum involved in the ultra-micro technique that the removal of the protein is unnecessary and might in fact cause an error in transferring etc.

Procedure:

POTASSIUM: Collect two capillary lithium oxylated tubes in the usual manner. Serum may be used if preferred, but extra care must be taken in rimming the clot to insure against homolysis. Pipette 0.05 cc. of clear plasma or serum into a 3 ml. tube and add 1.45 cc. of distilled water making the final dilution fifty times. Run with standards from 0.05 to 0.15 micro equiv. Na and K/cc. The settings of the Beckman are the same as for the macro method: Oxygen—16; Acetylene—3.0; sensitivity—complete clockwise, wavelength—766, selector switch—0.1; slit—0.5 and phototube—red.

SODIUM: Dilute the remainder of the serum left from the Potassium test, i.e., what remains in the testing cell, and dilute it 1 : 10 with distilled water. Example—if 0.8 cc. of diluted serum is left dilute it to 8.0 cc. This will make the final dilution 500 times. Run with standards varying from 0.2 to 0.3 micro equiv./cc. If Na is over 150 meq./1 run the 0.4 micro equiv. Na and K/cc. standard.* Plot both the potassium and sodium curves and read unknowns. Some laboratories do not believe in running the standards daily with their unknowns. It has been my experience that to do so is preferable and takes little extra time. It insures the fact that

* Na settings—O_2-15, Acet.—3, Sens. com. clock, W. L. 589, Sel. 0.1, Slit—.08, blue phototube.

you are reading the serum accurately and that the machine is not varying in any way due to a clogged burner, atmospheric conditions, battery variations, etc.

MICRO HEMATOCRIT: Heparinized capillary tubing is available for purchase if one does not wish to fix his own. Size 75 is the most desirable. Collect blood as described and seal with the wax in the described manner. Spin the capillary tubes in 3 ml. test tubes at 2,000 RPM's for 10 minutes.

To read take a mm. ruler and read the entire column. Be sure the ruler is level with the wax that is within the capillary tube. The wax may have to be chipped off the bottom in order to do this. A Bard-Parker blade may be used.

Next read just the column of red cells. Divide the red cell column by the length of the entire column $\times$ 100 = hematocrit reading.

A battery of tests may be run on as little as 0.05 cc. plasma or serum including Na, K, Protein and also Hematocrit if plasma is drawn. To do this one proceeds in the following manner:

Draw two capillary tubes approximately three inches long with lithium oxylate 1%. Fill the tube with oxylate and blow out leaving a small amount of liquid oxylate. Fill the tube with the blood sample from the oxylate end and shake oxylate bubble down three times with a snapping action of the wrist. Seal the end in melted wax by pushing the tube against the side of the dish as described in the beginning of the article. Spin at 2,000 RPM's for 10 minutes.

1. Measure hematocrit at this point as described under micro hematocrit.

2. File at junction of serum and column of red cells and expel clear serum into a 3 ml. tube. This will be plasma in the event that a hematocrit was desired.

3. If Na and K are requested set them up at this point in this manner. Remove 0.05 cc. plasma and add 1.45 cc. water. The resulting solution is a fifty times dilution. Run the K and on the completion of this test if a protein is requested remove 1.0 cc. or 0.5 cc. from the micro cell. If 0.5 cc. is used divide all solution added by two, i.e., biuret solution and final water dilution. Micro cuvets must be used for the reading of this test if 0.5 cc. of the diluted plasma is used. Pipette up the remaining solution from the K dilution and whatever the amount dilute it 1:10 as described under Sodium. The dilution is now 500 times. Hemolysed serum may not be used for the K determination. A little practice will be required in learning to take the readings swiftly yet accurately on the spectrophotometer using the flame attachment in order that the original sample will not be used up with the initial test.

Micro-technique Procedures

MICRO SUGAR:

Solutions: Tungstic Acid Solution (TAS)—To 8.0 cc. of distilled water, add 1.0 cc. of 2/3 N H_2SO_4 and 1.0 cc. of 10% Sodium Tungstate. Mix.

Standard: Make up a standard containing 0.1 mg. glucose in 1.0 cc. from the stock 1% glucose solution which is made up to 100 cc. with 0.25% benzoic acid.

Blood Sample: Add 0.1 cc. blood to 1.9 cc. TAS or 0.05 cc. blood to 0.95 cc. TAS. Mix and centrifuge. The tube or filtrate is carried with the technician and the blood added directly.

The Alkaline Copper Tartrate and the Phospho-molybdic solutions are made up according to the Folin-Wu directions.

Procedure—

1. Using micro sugar tubes graduated at the 6.25 cc. mark, number the tubes 1, 2, 3, and 4. 1 and 2 will be labeled unknown, 3, the blank and number 4 the standard.
2. Place 0.5 cc. of filtrate in each of tubes 1 and 2.
3. Place 0.5 cc. of distilled water in tube 3.
4. Place 0.5 cc. of dilute standard in tube 4.
5. To each tube add 0.5 cc. of alkaline copper tartrate and boil for 6 minutes. Cool. Add 0.5 cc. of Phosphomolybdic Acid to each tube. Shake.
6. Let stand for five minutes to develop color and dilute to the 6.25 cc. mark. Transfer to cuvets and read on the Beckman using wave-length 420 and the blue phototube. Selector switch 0.1, complete sensitivity and the slit set to blank.

Calculations: $\frac{\text{Density of unknown}}{\text{Density of Std.}} \times 200 = \text{Blood sugar in Mg.\%}$

NOTE—This is a capillary technique and the results should be noted and interpreted to this effect. Capillary values run higher than venous values.

MICRO UREA:

Solutions—1. Tungstic Acid Solutions—Transfer 20 ml. of 10% sodium tungstate to a 1 liter volumetric flask. Add about 700 ml. of distilled water. Add, with shaking, 160 ml. of 1/12 N H_2SO_4 and dilute with distilled water to 1 liter. Keep in refrigerator and do not use if cloudy.

2. Nessler's is made up according to Koch-McKeekin—dissolve 30 gms. of KI in 20 ml. of distilled water and add 22.5 gm. of I_2 to the solution. Shake until dissolved and then add 30 gms. of pure metallic mercury. Shake the mixture well, keeping it cool by holding under running tap water from time to time, until the supernatant liquid has lost the yellow color of iodine. Pour off from the undissolved mercury and test for the presence of excess iodine by adding a few drops to a little starch solution in a

test tube. If no blue color is obtained add iodine solution similar to that previously used, drop by drop until there is a slight excess of free iodine as indicated by the test with starch solution. Dilute to 200 ml., mix and pour into 975 ml. of accurately prepared 10% sodium hydroxide solution. Saturated NaOH has 1100 gms. NaOH/L and has actually 74 gms./100 c.c.

$$\frac{10 \times 100}{74} = 13.5 \text{ cc. to } 100 \text{ cc. is a } 10\% \text{ solution.}$$

3. Buffer Solution: 20 gms. of sodium acetate dissolved in distilled water and add 2 ml. of 10% acetic acid. Dilute to 100 ml. and mix.

4. Urease: This can be obtained commercially.

Procedure: Use three tubes graduated at the nine cc. and 10 cc. mark. Label blank, standard and unknown.

1. Place 9.8 cc. of TAS in a 15 ml. centrifuge tube. Prick finger with a Bard-Parker blade so that the blood flows freely and collect 0.2 cc. of blood. Introduce into the centrifuge tube and rinse pipette. Stopper and mix. Centrifuge at a high speed for five minutes.

2. Into a small test tube pipette 4 ml. of supernatant fluid marked Unknown.

3. Into the tube marked standard, pipette 1 ml. of urea nitrogen standard solution containing 0.015 mg. urea nitrogen/cc.

4. Make up a blank with 4.0 cc. of water.

5. To each tube add 4 drops of urease and 2 drops of buffer.

6. Digest for 10 minutes in a water bath between 45° and 50° C.

7. Dilute to the 9.0 ml. mark with distilled water. Add one cc. of Nessler's.

8. Read 10 minutes after adding Nessler's using Beckman wavelength 430, blue phototube, selector switch 0.1, sensitivity two complete turns counter clockwise (this will vary with machine) and the slit set by the blank. Tubes may be iced during the 10 minutes if clouding is a problem.

Calculations: Factor is $18.\frac{RU}{RS} \times 18 = \text{BUN mg\%}$.

MICRO BLOOD CHLORIDE:

Solutions—1. Tungstic Acid Solution—Refer to TAS in micro urea.

2. Mercuric Nitrate Solution: Dissolve about 1.5 gm. of mercuric nitrate (reagent grade) in about 500 ml. of distilled water with the addition of 40 ml. of N nitric acid. Transfer to a one liter volumetric flask and dilute to the mark with distilled water.

3. Indicator: Dissolve 100 mg. s- diphenycarbazone (Eastman

No. 4459) in 100 ml. of 95% ethyl alcohol. Store in the dark in a refrigerator. This indicator keeps about two months and should be discarded when its color becomes a cherry red.

4. Standard Sodium Chloride solution—Dissolve 0.5 gm. of dry reagent grade sodium chloride in distilled water and transfer quantitatively to a one liter volumetric flask. Dilute to mark with distilled water. One ml. of this solution contains 0.5 mg. of sodium chloride. The salt may be dried in a drying oven at 110° to 120° C. for at least fifteen hours. Five cc. should be titrated with the Mercuric Nitrate.

Procedure: Pipette 5.0 ml. of the standard chloride solution into a small porcelain dish or a 50 ml. Erlenmeyer flask and titrate against a white background. Add four drops of the indicator and titrate with mercuric nitrate until a pale violet color appears. Find factor by dividing 5.0 cc. of NaCl by the cc. of mercuric nitrate.

Unknown:

1. Place 9.8 ml. of TAS in a 15 ml. centrifuge tube. Prick finger with a lancet so that the blood flows freely. Using a 2.0 ml. pipette collect 0.2 cc. of blood and rinse into tube. Centrifuge at high speed for ten minutes.

2. Pipette 2.0 cc. of filtrate into a 50 cc. Erlenmeyer flask and proceed as a macro chloride adding four drops of indicator and titrating with mercuric nitrate with a one ml. pipette. Run in duplicate.

3. Schales method is based on a 1:10 blood filtrate. This modification is based on a 1:50 dilution. To calculate proceed in this manner.

ml. of mercuric nitrate $\times$ factor $\times$ 250 (dil. factor) $\times$ 5 $\times$ 100 = mg% of blood Cl^- expressed as NaCl.

MICRO PROTHROMBIN:

Procedure:

Materials: 1. 0.2 cc. Folin Micro Sugar Pipettes
2. 75 x 10 mm. test tubes or 1.0 cc. centrifuge tubes
3. Simplastin extract
4. 0.1 M Sodium Oxylate Solution

Method:

1. Into a 75 x 10 mm. test tube or a 1.0 cc. centrifuge tube place 0.2 cc. of 0.1 M Sodium Oxylate solution.

2. In the same manner as taking a hemoglobin from a finger puncture obtain 0.2 ml. of blood with a 0.2 ml. micro pipette and mix with the sodium oxylate solution in the test tube.

3. Centrifuge the oxylated blood and remove the plasma with a fine capillary pipette and place in a small test tube.

4. Into another small test tube pipette 0.1 cc. simplastin and place in a 37° C. water bath for a few seconds.

5. Add 0.05 cc. plasma to the simplastin tube and start stop watch immediately. Keep the tube in the water bath and shake lightly. Then by gently tilting the tube observe the formation of the clot. Stop the watch.

6. If possible take two tubes to duplicate the test.

PRECAUTIONS: 1. Undue squeezing of the finger must be avoided as some tissue fluid would then be mixed with the blood sample.

2. All other precautions as described under Quick's method must be adhered to, also.

BLOOD pH:

Procedure—Capillary tubes having a 1.5 mm. bore are used to collect the blood sample without the addition of any anticoagulant. Consequently, depending on the size of the hospital, the ability to conduct the entire test at the bedside of the patient is worth considering. The pH is done with a battery operated Beckman, model G, pH meter in our laboratory. The one drop electrode is used for the determination. Before the actual test is performed the pH meter is adjusted with a freshly prepared pH buffer 7.40 (cenco buffer). The electrode is washed with distilled water, dried and rinsed with the buffer before the adjusting is attempted. After similarly washing with distilled water and the electrode is carefully dried a control blood sample is taken. When approximately 0.05 ml. has entered the tube it is transferred to the electrode cup and read. The actual reading takes about one minute as the needle will drift until the blood comes to room temperature. The reading is recorded and the electrodes rinsed and dried as previously described. The patient's blood is now drawn into capillary tubing as described under the control sample and placed in the cup. The reading is taken over the one minute period until the needle ceases to fluctuate and the pH is recorded.

The pH of blood will vary approximately 0.015 units per degree drop, thus a sample of blood having a pH of 7.62 at 25°C has a corrected pH value of 7.44.

Calculation—Degree difference between 37° and 25° C = 12. $0.015 \times 12 = 0.18$. $7.62 - 0.18 = 7.44$.

TABLE 1
Micro Techniques

Procedure		Cap. Bl. pH	Pro-thrombin	Sug. mg. %	Urea mg. %	Bl. Chlor. mg. %
No. of Analysis		13	9	8	8	8
Average	Micro	7.50 corrected	14.2 sec.	90	11.6	521
	Macro		14.9 sec.	91.8	12.0	520

Both the control and the patient's corrected value should be reported.

TABLE 2
Ultra-Micro Tech.

Procedure		Hem. %	K meq/l	Na meq/l	Chlor. meq/l	Protein gm. %
No. of Analysis		6	10	10	6	15
Average	Ultra-mic	42	5.1	146	98.2	5.80
	Macro	42.8	4.52	144	98	5.79

Procedures for macro techniques performed on venous blood and micro and ultra-micro procedures on capillary blood.

Summary

Procedures are described for ultra-micro techniques concerning serum or plasma—chloride; protein; sodium; potassium and hematocrit. Also, micro-methods for blood—chloride; sugar; urea; prothrombin and pH. Methods for obtaining samples, techniques helpful in assuring accuracy and comparable data on results are also included.

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A RAPID MICRO METHOD FOR THE DETERMINATION OF BLOOD OXYGEN CONTENT, CAPACITY, AND PER CENT SATURATION*

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Accurate and rapid micro methods for determination of blood oxygen content, capacity, and, as a resulting calculation, per cent saturation are valuable clinical and research laboratory tools. They are especially important in pulmonary and cardiac function studies on children or small animals and negate the necessity for large blood samples in similar work on adults. Micro procedures are gasometric in which oxygen is measured by pressure[1] or volume[2] changes, and spectrometric[3, 4] in which differential spectral characteristics of oxyhemoglobin are analyzed in a spectrophotometer. The gaseous volumetric technique requires the least expensive apparatus and is the most practical for a clinical or small research laboratory.

Roughton and Scholander,[2] in 1943, developed a micro volumetric method for measuring oxygen content in 0.04 milliliters of blood. They used as an analyzer a graduated capillary tubing fused to the tip of a one milliliter tuberculin syringe. Oxygen and other blood gases were extracted by excess carbon dioxide. Carbon dioxide was absorbed with alkali and the amount of oxygen determined by measurement of gas volume before and after addition of oxygen absorbing solution. Lilienthal and Riley,[5] applying this procedure to physiologic studies, found the accuracy was less than that obtained by the Van Slyke manometric macro method.[6] Without increasing reliability, other authors[7, 8] have recently modified chemicals or apparatus employed in the technique.

Preston and Ordway[9] measured oxygen capacities as well as contents by the original Roughton-Scholander method. For oxygen capacity, 0.5 milliliter of blood was first aerated in a separate container and then tested. Later, Grant[10] utilizing the Roughton-Scholander syringe as aeration vessel and also analyzer, devised another technique for oxygen capacity. This procedure avoids the errors described by Roughton, Darling, and Root[11] which occur when blood is aerated separately.

To calculate per cent oxygen saturation of hemoglobin, oxygen content is divided by oxygen capacity. Both values are corrected for dissolved oxygen before the division is made. Oxygen saturation of venous blood varies; but the mean value for normal human arterial blood has been established as 98 per cent by several workers[12, 13, 14] using spectrometric and macro gasometric procedures. In contrast, Preston and Ordway,[9] conducting the only study of normal

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arterial saturation using micro techniques, found a mean value of 93 per cent in children.

It is the purpose of this paper to present modified micro volumetric methods for accurate and rapid determination of blood oxygen content and capacity, and to calculate by means of these methods correct values for normal arterial oxygen saturation.

MODIFIED METHODS

Oxygen Content—Modified Roughton-Scholander Method

Special Apparatus—1. Roughton-Scholander syringe† which is a 0.5 mm. precision bore capillary glass tubing fused to a one ml. tuberculin syringe. The capillary is 12 cm. in length and marked with 50 graduations or divisions. A cup at the tip of the capillary is 1.5 cm. long with a 2.5 mm. inside diameter. The manufacturer places a mark on the cup and a second mark is etched 5 mm. above the first. Pipets supplied with the syringe are of 0.04 ml. capacity and have tips ground to fit the bottom of the syringe cup. All pipets and syringes are, for practical purposes, of uniform volume. A rubber cup of one ml. volume is attached to the glass cup during one step of the technique.

2. Vernier-Viewer* which consists of a pocket microscope (40—60X magnification) attached to the movable arm of a vernier calipers. This apparatus can be obtained commercially or assembled inexpensively in the laboratory. The vernier-viewer is fixed to the side of a $37.5 \pm 0.2°$C. water bath. The viewer is foscused on a platform placed below the water level where syringes are equilibrated and read. A gas bubble length in the syringe capillary is measured by placing the hair-line in the eyepiece of the viewer at one meniscus of the bubble and noting the reading on the vernier. The viewer is then movd along the vernier to the other meniscus and the reading there recorded. The difference in the two readings is the length of the bubble.

Reagents—Solutions 1, 3, and 4 are renewed each day. The reagents are stored in syringes whose plungers have been lightly greased. Ten ml. capacity syringes are suitable for reagents 1, 2, and 3; five ml. syringes for 4 and 6; and a twenty ml. syringe for 5. Syringes containing solutions 2, 3, 4, and 6 are fitted with 26 gauge needles and 23 gauge needles are used for 1 and 4.

1. Ferricyanide solution: Mix together 12.5 gm. potassium ferricyanide, 4 gm. potassium bicarbonate, and 0.8 gm. saponin.** Dilute to 50 ml. with distilled water.

2. Acetate buffer: Dissolve 70 gm. sodium acetate in 100 ml. of distilled water and add 15 ml. glacial acetic acid.

† Macalaster Bicknell Co., 181 Henry St., New Haven, Conn.
* Respiration Aids, 424 E 123rd St., New York 35, N. Y.
** Saponin-Practical, No. P4794, Organic Chemical Division, Eastman Kodak Co., Rochester, N. Y.

3. Dreft solution: Make a saturated solution of commercial Dreft and distilled water.

4. Hydrosulfite solution: Dissolve 0.5 gm. sodium hydrosulfite in 2.5 ml. of 1N potassium hydroxide and filter through a small piece of cotton. Avoid exposure of the solution to air.

5. Sodium hydroxide: 10 per cent solution.

6. Caprylic alcohol.

Blood Samples:—Arterial and venous blood is drawn into 2 or 5 ml. capacity syringes with oiled plungers. Before sampling a small amount of heparin is drawn into each syringe. Then, air and heparin are expelled leaving a little heparin to fill the tip of the syringe. Samples are anaerobically taken from indwelling arterial or venous needles or catheters. A small quantity of chemically clean mercury is drawn into each blood sample to facilitate mixing. To seal the syringe a 2.5 cm. length of rubber tubing is attached to the nozzle; some blood is expelled through the tubing; and it is closed with a glass plug 2 cm. long. Samples are immediately placed in ice water and kept in the dark. In addition, blood from a finger prick may be drawn directly into a Roughton-Scholander blood pipet which has been rinsed with heparin and air dried. The sample is then analyzed immediately.

Procedure—1. Rinse the Roughton-Scholander syringe thoroughly with detergent solution, tap water, and, finally, distilled water.

1. Hold syringe vertically and remove any liquid in cup with a needle attached by rubber tubing to a suction bottle.

3. Fill cup with ferricyanide solution; draw into syringe; expell into cup and remove. Repeat twice with fresh ferricyanide. No air bubbles are present in syringe or capillary.

4. Fill cup with ferricyanide solution to the second or upper mark and draw to the bottom of cup.

5. Place a drop of caprylic alcohol in the bottom of cup.

6. Clamp syringe to a ring stand with cup raised at a 45° angle from the horizontal.

7. Mix blood sample well and remove glass plug. Expell a few drops of blood through the rubber tubing and placing it snugly against the blood syringe nozzle, advance syringe plunger and blood will flow smoothly up to the 0.040 ml. mark on the pipet. Wipe excess blood from pipet.

8. Insert blood pipet into cup of Roughton-Scholander syringe and press it tightly against cup bottom. With the opposite hand pull back slowly and steadily on syringe plunger.

9. After all blood has entered the syringe, a bubble of air 1 mm. in length is drawn into cup. Remove pipet and immediately expell this bubble of air by advancing plunger slightly or tapping capillary.

10. Draw in a trace of caprylic alcohol. A drop 1 mm. in length

as measured in the capillary is sufficient. Remove remaining caprylic alcohol.

11. Fill cup with acetate buffer to the second or upper mark and draw this to bottom of cup.

12. Fill cup at once with Dreft solution and firmly close cup with the index finger.

13. Loosen the arresting clip on syringe plunger and shake syringe vigorously in a horizontal position for 2 minutes. As gases are evolved plunger will move out. Plunger may have to be twisted out a little at first to keep it from sticking to syringe barrel. The total volume of gas should be 0.75 ml. If this amount of gas is not evolved, the strength of the bicarbonate should be adjusted.

14. Remove finger slowly so as to keep gas bubble from escaping. Hold syringe vertically and draw a small amount of Dreft solution into capillary. Replace plunger arresting clip.

15. Remove excess Dreft from cup. Fit rubber cup over glass one and fill former with 10 per cent sodium hydroxide.

16. Draw a little sodium hydroxide into syringe. Absorption of carbon dioxide will be rapid and remaining bubble is quickly and evenly pushed up into capillary by manipulation of plunger.

17. Remove rubber cup and empty glass cup. Place syringe on the reading platform in the water bath.

18. While temperature equilibration is taking place, repeat steps 1-17 using another syringe for duplicate analysis.

19. By means of the vernier-viewer measure length of bubble, L1, in first syringe.

20. Take first syringe from bath and hold in a vertical position. Fill the cup with hydrosulfite solution. Oxygen is absorbed by moving the bubble up and down 3 or 4 times from top to bottom of capillary. Remove excess hydrosulfite from cup and place syringe in water bath for second temperature equilibration.

21. Carry out steps 19 and 20 on second syringe.

22. Measure length of residual bubble, L2, in first syringe and then second syringe.

23. Wash syringes as in step 1. Never force blood precipitates into capillary.

24. Duplicate blank determinations are carried out each day by repeating the above tests without the addition of blood. This blank value indicates amount of oxygen dissolved in reagents.

25. Record barometric pressure each day.

Oxygen Capacity—Modified Grant Method

Reagents—In addition to those reagents listed above, isotonic sodium chloride is needed.

Procedure—1. Fill Roughton-Scholander syringe cup with

saline and draw into syringe. Expel and repeat twice with fresh saline. Lastly, add saline to the lower or manufacturer's mark on cup.

2. Carry out steps 6, 7, and 8 of oxygen content procedure.

3. Draw saline which remains in cup into syringe and follow it with approximately one ml. of air.

4. Place syringe on a mechanical rotator and rotate horizontally for 3 minutes. Occasionally, rotate it by hand in a vertical position to spread the blood throughout the syringe and expose all of it to air.

5. Once a minute during rotation, expell air in syringe and draw in a new sample. Do not lose any blood during this operation. A trace of caprylic alcohol in the capillary is often helpful.

6. Expell third sample of air and run blood up to the bottom of cup. No air bubbles should be present in capillary.

7. Carry out step 4, and steps 10-17 of oxygen content method.

8. While temperature equalibration of gas bubble is taking place, repeat steps 1-5 of this procedure using another syringe for duplicate analysis.

9. During rotation of second syringe, carry out steps 19 and 20 of content method on first syringe.

10. Repeat steps 6 and 7 of this technique on second syringe.

11. Measure length, L2 of residual bubble in first syringe.

12. Measure L1 for second syringe; absorb oxygen; equilibrate residual bubble in bath; and measure L2 as above.

13. Duplicate blank measurements are made each day by replacing blood with saline in the pipet and repeating above procedure. This blank value will include oxygen dissolved in the reagents as well as that in the blood.

Time—Duplicate determinations require approximately 20 minutes for oxygen content and 26 minutes for oxygen capacity.

Calculations—The formula for oxygen capacity is the same as that used to calculate oxygen content. The vernier calipers used in these experiments read in inches. Adjustments in the formula can be made when a calipers reading in centimeters is employed.

The formula is:

$$\text{Volumes per cent oxygen} = \frac{(L1 - L2 - B) \times S \times F}{P} \times 100$$

Where:

L1 and L2 are gas bubble lengths before and after oxygen absorption. These are read in inches to the third decimal place, for example, 2.607 inches.

B is the value for oxygen dissolved in the reagents. B for oxygen capacity also includes oxygen dissolved in the blood as well as reagents.

S is the volume of the syringe capillary per inch of length. S = 0.00514 ml./inch.

F = the factor which converts gas bubble volume to standard conditions.

$$F = \frac{273 \times BP - 47}{273 + 37.5 \times 760}$$

BP — 47 is the observed barometric pressure minus the water vapor pressure at 37.5°C. which is 47 mm. Hg.

Values for F at various barometric pressures are given in Table 1.

P is the volume in milliliters delivered by the blood pipet. P varies slightly with different pipets; but for most purposes a value of 0.0403 ml. is suitable. For highly accurate work pipets should be individually calibrated.

Finally, the formula simplifies to:

Volumes per cent oxygen = (12.8F) (1.1 — 1.2 — B)

The formula for per cent saturation is:

$$\text{Per cent oxygen saturation of the hemoglobin} = \frac{\text{Oxygen content of the hemoglobin in vols. \%} - \text{Dissolved oxygen in vols. \%}}{\text{Oxygen capacity of the hemoglobin in vols. \%} - \text{Dissolved oxygen in vols. \%}} \times 100$$

As mentioned above, when the blank value for oxygen capacity is substracted from the blood oxygen capacity, no further correction is needed for physically dissolved oxygen. Conversely, dissolved oxygen must be subtracted from the blood oxygen content as measured by the method of this paper. Values for dissolved oxygen vary from 0.3 volumes per cent for normal arterial blood to 0.1 volumes per cent or less for venous blood.[15]

TABLE I

Factors to Reduce Gas Bubble Volume to Standard Temperature and Pressure—Dry.

BP—47	F	BP—47	F
650	0.7520	705	0.8157
655	0.7578	710	0.8215
660	0.7636	715	0.8275
665	0.7694	720	0.8330
670	0.7752	725	0.8388
675	0.7810	730	0.8446
680	0.7868	735	0.8504
685	0.7925	740	0.8562
690	0.7983	745	0.8620
695	0.8041	750	0.8677
700	0.8099		

BP = Observed barometric pressure in mm. Hg.
47 = Water vapor pressure at 37.5° C.
F. = Factor by which to multiply gas bubble volume.

EXPERIMENTAL METHODS

Modifications

Apparatus—Volumes of six blood pipets and two Roughton-Scholander syringe capillaries were measured by gravimetric calibration with mercury. The relationship of volume in milliliters to length in inches was determined for each syringe capillary.

New apparatus adopted were the vernier-viewer and the constant temperature water bath. In addition, a second mark was

etched on each syringe cup 5 mm. above the first or manufacturer's mark.

Reagents—The amount of saponin was increased from 0.5 to 0.8 gm. Forty-five per cent urea was replaced by saturated Dreft solution. Hydrosulfite solution was used as oxygen absorber instead of pyrogallol.

Technique—Changes in procedure were the: simultaneous analysis of duplicate samples, loading of the blood pipet, and delivery of blood into the Roughton-Scholander syringe. Larger volumes of reagent solutions were also employed. These were measured at the second or upper mark on the cup.

Modifications in the oxygen capacity technique included those applied to the oxygen content procedure and, in addition, the time of blood aeration was shortened from 5 to 3 minutes. The optimum time for complete hemoglobin aeration was established by a series of experiments. Finally, slightly less saline was used for the blank than in the original method.

Calculations—A new formula for calculation of oxygen content and capacity was derived, as well as a table of figures to reduce gas volumes to standard conditions.

Accuracy and Precision

The validity of the micro oxygen content and capacity methods was established by comparisons with widely recognized macro methods.

Thirty-two blood samples were analyzed for oxygen content by the micro techniques presented here and the macro manometric procedure of Van Slyke and Neill.[6] In the latter method, a neutral ferricyanide-saponin solution releases oxygen, carbon dioxide, and other gases from blood under vacuum. Carbon dioxide is absorbed by alkali and the amount of oxygen determined by pressure measurement before and after its absorption with hydrosulfite.

Oxygen capacities of 18 samples were determined by the micro technique of this paper and the macro manometric chamber method which is the Roughton, Darling, and Root[11] modification of Sendroy's procedure.[16] In their method, blood is saturated with oxygen in the chamber of the Van Slyke apparatus by mixing with saline previously saturated with oxygen. Oxygen combined with the hemoglobin is then analyzed as for oxygen content. Duplicate determinations by the content and capacity macro methods agreed within 0.1 to 0.15 volumes per cent or less.

The precision of each of the new methods was measured by recording the differences in 60 duplicate analyses for oxygen content and 40 duplicate analyses for oxygen capacity.

Normal Values for Arterial Blood Oxygen Saturation

The normal arterial oxygen saturation of men was established

for the micro methods by comparison to macro gasometric values. Eleven normal men between the ages of 20 and 50 were studied. Normal arterial blood was considered to contain 0.3 volumes per cent of physically dissolved oxygen[15] which was subtracted from each oxygen content. Correction for dissolved oxygen in the oxygen capacity values were made as described by Roughton et. al.[11]

EXPERIMENTAL RESULTS

Modifications

Apparatus—six pipets were calibrated gravimetrically in duplicate and the mean value was 0.0403 ± 0.0002,* with a range of 0.0401 to 0.0406 ml. Two Roughton-Scholander syringe capillary volumes were calibrated in duplicate and found to be identical. Each capillary contained 0.000392 ml. for a 0.0762 inch length, which was the mean width of each division or graduation marked on the capillary by the manufacturer. The widths of 45 separate divisions were measured with the vernier-viewer and the mean value was 0.0762 ± 0.0005, with a range of 0.0756 to 0.0770 inches. Therefore, the volume of each capillary was 0.00514 ml./inch. The volumes of syringe capillaries and pipets are recorded in Table II.

TABLE II

Volumes of Syringe Capillaries and Blood Pipets, Calibrated Gravimetrically in Duplicate.

Syringe Capillaries	Volume in Milliliters Inch
1	0.00154
2	0.00154
	mean = 0.00154
Pipets	**Volume in Milliliters**
1	0.0404
2	0.0401
3	0.0406
4	0.0402
5	0.0402
6	0.0405
	mean = 0.0403 ± 0.0002

The use of the vernier-viewer permitted accurate and precise readings of gas bubble lengths in the syringe capillary. Bubbles were originally measured by the unaided eye from graduations on the syringe capillary. The vernier-viewer has been employed to avoid the errors of parallax caused by the former estimation of bubble lengths.

Equilibration of bubbles in a constant temperature water bath and the subsequent measurement of their lengths at constant temperature negated errors due to gas volume changes with temperature. Previously, bubbles were equilibrated in a beaker of water at room temperature, removed, and read in the air.

* Standard Deviation of the Mean.

Reagents—The increased amount of saponin was found necessary to insure optimum hemolysis in a few seconds. A brand of saponin with strong hemolytic properties was used. Blood precipitates were cleared from the capillary more rapidly by saturated Dreft solution than by 45 per cent urea. In addition, bubbles did not break up in the capillary as they did when urea was employed. Hydrosulfite solution was a rapid oxygen absorber and more convenient to use than pyrogallol. If reagent grade sodium hydrosulfite is used, no undesirable effects, as described by Roughton and Scholander,[2] are encountered.

Technique—The various steps of these micro methods have been simplified so that they were easily mastered. Duplicate analyses were made in 20 minutes. Loading of the blood pipet and delivery of blood into the Roughton-Scholander syringe has been changed to avoid the difficulty with these steps as given in the original method. Reagent solutions are filled to the upper or etched mark on the cup to insure the necessary amount of chemicals for optimum release of oxygen. Before, solutions were filled to the lower mark and, as a result, low values were obtained on bloods with abnormally high oxygen contents or capacities.

For use in the oxygen capacity method, the necessary length of time for blood aeration was established by a series of experiments in which blood samples were aerated in the Roughton-Scholander syringe for 3 and 5 minutes. The diluting saline was unoxygenated or oxygenated by passing 100 per cent oxygen through it. The air volume was replaced three times in all experiments. After aeration the oxygen capacity was determined as described above. Blank values for dissolved oxygen were determined in each case. The results of these experiments are shown in Table III. It was found that the optimum time for aeration was three minutes and the addition of oxygenated saline was not needed if the air volume was replaced once a minute. In the original method blood was aerated for 5 minutes.

TABLE III

Time Needed for Complete Hemoglobin Oxygenation in the Micro Oxygen Capacity Method.

Blood Sample No.	Diluted with Unoxygenated Saline Vols. % O_2	Diluted with Oxygenated Saline Vols. % O_2	Time of Aeration* Minutes
1	17.57	17.60	5
	17.73	17.68	3
2	20.36	20.27	5
	20.36	20.32	3

* Air volume in syringe was replaced three times in all experiments.

Lastly, for further application to the oxygen capacity method,

the amount of saline used in the blank was studied. The oxygen solubility coefficient for saline was inferred from Sendroy's work[15] to be, for practical purposes, equal to that for whole blood. Therefore, 0.0403 ml. of saline was used in the blank to determine the amount of physically dissolved oxygen in the blood sample. Previously, it was thought necessary to employ 10 per cent more saline in the blank.

Calculations—Utilizing the results of pipet and syringe volume calibrations, a new formula was derived for calculating volumes per cent oxygen content or capacity. Reduction of gas volumes to standard conditions was simplified by means of Table 1.

Accuracy and Precision

The results of the analysis of thirty-two blood samples for oxygen content by the micro technique of this paper and the macro procedure of Van Slyke and Neill[6] are recorded in Table IV. Oxygen contents varied from 6.47 to 23.00 volumes per cent. The mean of the differences, micro minus macro, was -0.02 ± 0.14, with a range of -0.29 to $+0.26$ volumes per cent. The mean difference was not significantly different from zero, $t = 0.7952$ and $p > 0.4$.[17]

Oxygen capacities of 18 blood samples determined by the micro technique presented above and the macro method of Roughton and associates[11] also showed close agreement. The results are given in Table IV. Oxygen capacity values varied from 14.29 to 24.02 volumes per cent. The mean of the differences, micro minus macro, was -0.03 ± 0.13, with a range of -0.30 to $+0.14$ volumes per cent. The mean difference was not significantly different from zero, $t = 0.9693$, and $p > 0.3$.

TABLE IV

Accuracy of Micro Volumetric Methods, Determined by Comparison With Macro Manometric Techniques.

Method	Number of Comparisons	Range of Values Vols. % O_2	Mean Difference Micro-Macro Vols. % O_2	Range of Differences Vols. % O_2	P
O_2 Content	32	6.47 to 23.00	-0.02 ± 0.14	-0.29 to $+0.26$	<0.4*
O_2 Capacity	18	14.29 to 2402	-0.03 ± 0.13	-0.30 to $+0.14$	0.3*

* Differences not significantly different from zero.

Precision of the micro method for oxygen content was determined in 60 duplicate analyses and is shown in Table V. The range of contents was 5.04 to 24.60 volumes per cent. The mean of the differences between duplicates was 0.09 ± 0.09, with a range of 0.00 to 0.30 volumes per cent. The results of 40 duplicate measurements of oxygen capacity are also given in Table V.

TABLE V
Precision of Micro Volumetric Methods.

Method	Number of Duplicate Measurements	Range of Values Vols. % O_2	Mean Differences Vols. % O_2	Range of Differences Vols. % O_2
Oxygen Content	60	5.04 to 24.60	0.09 ± 0.09*	0.00 to 0.30
Oxygen Capacity	40	13.09 to 26.45	0.11 ± 0.07	0.01 to 0.24

* Standard deviation of the mean.

The range of capacities was 13.09 to 26.45 volumes per cent. The mean of the differences between duplicates was 0.11 ± 0.07, with a range of 0.01 to 0.24 volumes per cent.

Normal Values for Arterial Blood Oxygen Saturation

The excellent agreement between the new micro volumetric techniques and the macro manometric methods permitted establishment of a normal range of arterial oxygen saturation by the micro procedures. The arterial bloods of 11 normal men between the ages of 20 and 50 were previously analyzed by the macro determinations described above. The mean value was 98, with a range of 96 to 100 per cent oxygen saturation. This range was also assigned to the micro procedures.

DISCUSSION

These modified micro volumetric procedures for oxygen content and capacity are as accurate as the usual macro manometric methods. They are simpler to learn and require less expensive apparatus than macro or other micro procedures. Time involved is half that needed for a macro analysis. Another advantage is that duplicate analyses for content and capacity can be made on less than a milliliter of blood. An eight milliliter sample, at least, would be required for a macro determination.

The normal arterial oxygen saturation of children was not measured; however, there is no physiological basis for believing they are more unsaturated than adults. The value of 93 per cent for children, obtained by Preston and Ordway,[9] is 5 per cent lower than the established adult value. This discrepancy is no doubt due to falsely high oxygen capacity results which are principally caused by concentration of red cells when aeration of blood takes place in a separate container from the one in which analysis is carried out.[10,11] The micro method presented here is considered to give a more true value for oxygen capacity, and as a result oxygen saturation, than the Preston and Ordway procedure.

SUMMARY

Accurate and rapid micro volumetric methods for the determination of blood oxygen content, capacity, and per cent saturation have been presented in detail. A blood sample of 0.04 milliliters volume was required for each measurement. Duplicate analyses were completed in 20 minutes. The micro procedures were simple to learn and inexpensive. Accuracy and precision

were found to be equivalent to macro manometric techniques. The mean value for normal arterial oxygen saturation by micro methods was established as 98, with a range of 96 to 100 per cent.

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INTEREST TO SMALL HOSPITAL LABORATORIES IN REGARD TO MICROTECHNIQUE IN BLOOD CHEMISTRY*

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Microtechniques are essential in the modern laboratories and hospitals for many reasons. They make possible the obtaining

of quantitative laboratory information where larger amounts of blood are not available, such as occurs in acutely ill infants or special cases, as when extensive burns make venipunctures difficult. The data places the diagnosis and treatment of the results of such problems on the same basis as that of the macrotechniques. They make possible a wide variety of determinations on one small sample so that all chemical aspects of the disease may be cited if desired. They make possible repeated analyses so as to aid in securing diagnosis, treating and in following the course of disease. For example, following subsequently bilirubin in the blood by means of the Van den Berg test where blood incompatabilities exist. Determination of sugar in the blood may be done repeatedly to evaluate insulin requirements, or other tests requiring repeated analyses may be done on finger tip blood.

As a rule, micro methods are as rapid or faster than macro methods. This is usually the case because micro technologists from the beginning have stressed avoidance of elaborate procedures to eliminate contamination which is more serious when microprocedures are used. Specific micro instruments have been designed to obtain speed and simplicity for this purpose.

The main development of microchemistry is due primarily to volume reduction. In certain patients and especially in children venipunctures are undesirable. Since only 0.2 to 0.3 cc of capillary blood can be obtained readily from the finger or heel, it is necessary to perform chemistry tests on micro quantities of blood within the range of 0.1 to 0.3 cc.

In evaluating comparisons of micro and macro technique in chemistry, I have concentrated on tests which would be of most interest in pediatric diagnosis.

I have been successful in developing blood sugar, Van den Berg and inorganic phosphorous micro techniques within a 2% comparison with the macro techniques. The methods were evaluated by using a standard macro procedure in comparison with a micro procedure. However, comparative results of the micro Van den Berg in relation to the macro procedure are not evident until the bilirubin content of the blood has risen to 5 mg/100 cc of blood.

In evaluation of these three tests, I was not in access of micro-pipets, micro-kletts, or other special instruments which have been adapted for micro work. Instead, I developed these three procedures using equipment which is available in all clinical laboratories and hospitals. The equipment is essentially the same which I used for my macrotechniques. Therefore, I feel that these tests may be of value to these laboratories or hospitals which do not have equipment for micro work, but which

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June 1956.

do have the problems of blood collection.

In the determination of sugar, bilirubin, and inorganic phosphous, the blood used is between 0.1 to 0.2 ml. and is measured by using a 0.2 ml. pipet. The other solutions used in the procedures are measured with the ordinary laboratory lcc, 5cc and 10cc pipets. All the results of the tests were read on the Klett-Summerson Colorimeter. Micro sugar tubes are used in the sugar determinations which are calibrated to 6.25 cc.

DETERMINATION OF SUGAR

1. 0.1 ml. of blood is washed in 0.7 ml. of H_2O.
2. 0.1 ml. of 2/3 N H_2SO_4 and 0.1 ml. of 10% H_2WO_4 are added to precipitate proteins.
3. The mixture is centrifuged for 5 minutes.
4. 0.5 ml. of supernatant fluid is placed in a micro-calibrated test tube.
5. 0.5 ml. of Alkaline Copper reagent is added and the solution is heated at 100° for 6 minutes.
6. After cooling for 3 minutes, 0.5 ml. of Phosphomolybdic acid is added.
7. Dilute to 6.25 cc. with H_2O and mix.
8. The standard is prepared by adding 0.5 ml. of sugar standard (2cc = 2 mg.) and proceeding with step 5.
9. Read with blue filter on Klett-Summerson Colorimeter.

Calculation: U/S x 100 = mg of sugar /100 cc blood.

DETERMINATION OF BILIRUBIN

1. To direct and direct blank add 2.5 cc. H_2O.
2. To total and total blank add 2.5 cc. methyl alcohol.
3. To direct blank and total blank add 0.5 cc. HCl blank.
4. To direct and total add 0.5 cc. Diazo reagent.
5. Dilute patients serum 0.2 ml. with 1.8 cc. H_2O.
6. Add 0.5 cc. of diluted serum to each tube.
7. Dilute all tubes up to 5.0 cc. with H_2O.
8. Let stand 30 minutes and read with green filter on Klett-Summerson Colorimeter.

Calculation:

Reading of total X .07 X 4 minus reading of direct X .07 X 4 = Indirect.

Direct + Indirect = Total ml. of bilirubin /100 cc. of blood.

*Diazo reagent (10 ml. sulfanilic acid + 0.3 ml. of 0.5% sodium nitrite).

INORGANIC PHOSPHOROUS

1. Wash 0.2 ml. serum into 1.8 ml. 10% trichloroacetic acid.
2. Centrifuge. To 1.0 ml. of supernatant, 0.2 ml. of acid molybdate is added and mixed.
3. 0.1 ml. of Phosphomolybdate Reducing reagent (aminoaptholsulfonic acid reagent).
4. The standard is prepared by adding 1 ml. of phosphate standard (5cc = .025 mg.) and is diluted up to 5.0 ml. of H_2O.
5. The blank is prepared by adding 1 ml. of 10% trichloroacetic acid and is diluted up to 5.0 ml. with H_2O.
6. Dilute unknown to 5.0 ml. with H_2O, and read standard, blank, and unknown on thick red filter on Klett-Summerson Colorimeter.

Calculation:

U/S X 5 = ml. of inorganic phosphorous /100 cc. blood.

Summary

The operation of blood sugar, Van den Berg, and inorganic phosphorous microtechniques, using equipment which is available

to all hospitals and laboratories is described. Included is a discussion of the necessary equipment, and procedures for performing the three common analyses. With each method requiring only 0.1 ml. to 0.2 ml. of blood, a 2% comparative evaluation with the standard macro procedures is given.

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EXPERIENCES IN THE USE OF ULTRAMICRO METHODS IN ROUTINE CLINICAL CHEMISTRY

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During the past few months successful ultramicro methods in the evaluation of blood serum and urine of burned patients has prompted us to publish this paper with the hope that technologists now using only the macro method of analysis will come to appreciate the advantages of ultramicro determination. In this series of burned patients we carried out an average of 14 daily determinations on serum and urine which included the following: sodium; potassium; chloride; carbon dioxide; total protein; albumin; globulin; non-protein nitrogen; total nitrogen; pH; and hematocrit. After an average of about 140 determinations per burned patient, we have reached the conclusion that by comparison to the macro method, ultramicro methods offer additional advantages not commonly taken into consideration. Among these are conservation of reagents, versatility, conservation of time by the elimination of pipetting large amounts and the use of capillary puncture rather than venous puncture. It goes without saying that the use of capillary blood is of great advantage to technologists doing work on infants and small children. No significant differences in results were noted at any time between the ultramicro and the macro methods. We cannot fail to stress the importance of employing the routine procedure of running "recoveries" to check the accuracy of any analytical method, especially the ultramicro method. In our hands it has proven satisfactory both as far as time is concerned and, especially, in the satisfaction derived from knowing the accuracy and limitations of each determination.

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* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

Procedure

The ultramicro methods employed in our laboratory are basically those described by Samuel Natelson[1] with a few changes necessitated by the use of our model Beckman DU Flame-Spectrophotometer and our desire for more accurate titration end point.

1. In the potassium determination it was necessary to modify the serum dilution from 1:250 to 1:100.
2. The procedure for total protein was not that of Natelson but a method devised by Howe[2] which includes the determination of albumin.
3. In determining chloride, we preferred to use a 1:50 rather than 1:100 dilution in order to ensure a sharper end point.

Recoveries were run on all the above mentioned procedures routinely. The amount added was 30% or more of the unknown sample. Table 1 below indicates an analysis of a few of the determinations run and includes the percentage range of recovery and the average recovery for the stated number of determinations.

TABLE 1

	Unknowns	Recoveries	Total Samples	Percentage Recovery Range
Sodium	100	100	200	96—104
Potassium	100	100	200	96—104
Chloride	82	82	164	96—103
Carbon Dioxide	36	36	72	96—105
Total Protein	44	44	88	96—100
Nitrogen	37	37	74	98—102

Discussion

By employing the ultramicro method we have found that by making up only 50 cc. of reagent in, for instance, the total protein determination, it is possible to run as many as 500 tests as compared with the macro method in which only 10 determinations could be done with the same amount of reagent. While the technologists using the macro method require between 10-15 ml. of blood, we are able to run all these determinations, including recoveries, on 1 cc. of whole blood. This is of special consideration in patients in whom daily determinations for complicated electrolyte problems have to be carried out. It is of special advantage in infants in whom large volumes of blood loss are not only poorly tolerated but very difficult to obtain. Ordinarily, enough blood can be obtained from a finger or heel puncture for all the above-mentioned determinations. However, in this particular study of children venous punctures were used because of the extreme degree of subcutaneous edema regularly noted in severely burned patients. It goes without saying that it

is usually much easier to do a capillary puncture on an infant than to draw a venous specimen. It will be surprising to one who has been accustomed to pipetting with large amounts, how much time can be saved in using only 0.02 ml. or 0.2 ml. pipettes. Occasionally, we resorted to the use of .01 ml. and .1 ml. pipettes. Anyone familiar with the use of a flame-photometer will appreciate the fact that only small amounts of sodium-free and potassium-free water are required, thus eliminating the problem and expense of obtaining chemically pure water. Our procedures for "recoveries" were largely the result of trial and error, but the result obtained supplied conclusive evidence of the validity of these techniques since, in every recovery, we were able to obtain an accuracy of from 97 to 100 percent.

We preferred using Howe's method in our protein determination, since by salt-fractionation we could obtain additional information on the albumin-globulin partition. The total protein was checked by micro-Keldjhal determination and found to be accurate. Although our experiment has been running for a comparatively short time, we feel that the total number of determinations in each category has been enough to warrant sharing our experience with other technologists.

Acknowledgment

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Summary

Experiences in ultramicro methods of analyzing blood and serum of burned patients in a series of approximately 700 determinations demonstrated the advantages over conventional macro methods. These included conservation of time, material and patients' blood and, at the same time, proved accurate as seen by recovery studies.

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MICRO DETERMINATION OF BLOOD UREA NITROGEN

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Urea nitrogen is an important non-protein-nitrogenous constituent of blood and in health, it comprises about half its total. Inasmuch as urea is primarily affected in disease conditions associated with nitrogen retention, its determination is a more sensitive index of renal dysfunction than is the determination of

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non-protein-nitrogen. For example, the retention of 5 mgs./100 ml. of nitrogen would represent about 13-41% rise in the urea nitrogen, yet only a 1-2% rise in the total non-protein-nitrogen. The urea nitrogen determination of urea may also be of consequence in instances of liver disease where the ability to metabolize amino acids are impaired.

We believe the following micro procedure is the first of its kind using urease.[1] Gunderson and Rawlings[2] used diacetyl monoxine.

The principle used in the micro determination described here is the same as in the macro determination, that is, it relies upon the conversion of urea to ammonium carbonate by the action of urease on hemolyzed blood; the proteins together with the excess urease are precipitated by the addition of N/3 H_2SO_4 and 10% Na_2WO_4 and after Nesslerization, the intensity of the resulting yellow color is measured in a spectrophotometer at 500 mu.

Materials

Urease: Commercially prepared urease tablets containing 0.1 mg. urease per tablet are crushed and 1 ml. water is added per tablet.

N/3 Sulfuric acid: 5 ml concentrated H_2SO_4 are diluted to 500 ml with distilled water. Check by titration with standard alkali and adjust if necessary.

10% Sodium tungstate: Dissolve 10 grams of sodium tungstate in 100 ml. distilled water.

Nessler's reagent: Nessler's tablets are used.

All reagents must be especially low in ammonia content.

Calibration Curve

The calibration curve is made by using a standard solution of ammonium sulfate. The sulfate standard can either be dried and weighed out accurately and diluted with 0.1 N H_2SO_4 or a commercially prepared standard may be bought and diluted with the acid. 0.5, 1.0, 2.0, 3.0, 4.0 ml. of this 2 mg.% standard are pipetted into 25 ml. volumetric flasks. Distilled water is added until the flask is about half full. Two and one-half ml. Nessler's is added to each flask and then filled to the 25 ml. mark with distilled water. The equivalent concentrations of blood urea are plotted against per cent transmission on semi-logarithmic graph paper. Taking into consideration the amount and dilution of blood in this precedure, the five standards represent 4.0, 6.0, 16.0, 24.0, and 32.0 mgs.% urea nitrigen respectively for 100 ml. whole blood. The blank is prepared with 2.5 ml. Nessler's diluted to 25 ml. with distilled water.

Procedure

Wash 0.2 ml. of peripheral blood into 1.2 ml. of distilled water. Add 0.01 ml. of urease solution, stopper and incubate 15 minutes at 50°C. Add 0.4 ml. of N/3 H_2SO_4. Mix immediately and thor-

oughly. Add 0.2 ml. of 10% sodium tungstate, shake, wait 5 minutes and centrifuge. To 0.5 ml. of the supernatant add 4.0 ml. of distilled water and 0.5 ml. of Nessler's solution. Mix and read the percent transmittance at 500 mu. The percent urea nitrogen may be read directly from the calibration curve. A control is run simultaneously.

With care, the percent error in the micro method is comparable to that of the macro determination. In a typical comparative study, ten bloods were run using the macro and micro methods simultaneously. The results are illustrated in Table I.

TABLE I

Blood Specimen Number	Macro Determination	Micro Determination	Difference
1	16.0 mgs. %	17.0 mgs. %	+1
2	14.0 mgs. %	14.0 mgs. %	0
3	12.0 mgs. %	11.0 mgs. %	−1
4	18.0 mgs. %	17.0 mgs. %	−1
5	11.0 mgs. %	10.0 mgs. %	−1
6	8.0 mgs. %	9.5 mgs. %	+1.5
7	13.5 mgs. %	13.5 mgs. %	0
8	11.0 mgs. %	12.0 mgs. %	+1
9	9.5 mgs. %	9.5 mgs. %	0
10	14.0 mgs. %	14.0 mgs. %	0

Numbers 1-5 are determinations on sequestrenized venous blood.
The macro determinations on 6-9 are on sequestrenized venous blood while the micro determinations are on peripheral blood from the same patients.
Number 10 is the control.
Normal Range: 12-18 mgs. urea nitrogen/100 ml. blood.

Sources of Error

A possible major source of error is the contamination of the reagents with ammonia and for this reason it is desirable to run a blank on each lot of urease tablets. To do this, incubate 1 ml. of distilled water with 0.05 ml. of urease at 50°C for 15 minutes. Make a Folin-Wu filtrate by adding 8.0 ml of 1/12 N H_2SO_4 and 1 ml. of 10% Na_2WO_4; mix and filter through a No. 2 Whatman filter paper. Treat the filtrate as the micro supernatant. If a yellow color does appear after Nesslerization this should always be used in place of the water and Nessler blank previously described in the procedure. When sodium tungstate is added to the blood, the mixture should be allowed to stand 5 minutes before centrifugation. Using this precaution, cloudiness has not been observed.

Summary

A micro method for determination of blood urea is presented. It has proven useful as a routine clinical procedure in pediatric patients and on individuals who have inaccessible veins.

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PREVENTION OF LABORATORY-ACQUIRED INFECTIONS*

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Prevention of laboratory infections is principally a matter of preventing unrecognized respiratory inhalation of infectious microorganisms. I stress the word "unrecognized," because analysis of our experience at Detrick, as well as analysis of the published accounts of laboratory infections, shows that only about 16 to 20 per cent of the total infections can be attributed to known accidents.[1]

The number of illnesses reported for each disease is the result of a combination of factors, such as, infectiousness of the organism, number of persons potentially exposed, thoroughness of medical investigation of illness in laboratory personnel, completeness of reporting, and precautions taken. For instance, tularemia, Q fever, brucellosis and Rift Valley Fever are all easily acquired in the laboratory, but there are very few persons working with the latter, so only a few cases have appeared. On the other hand, many are exposed to tuberculosis in the laboratory and infection is not uncommon, but it is my impression that these cases are not freely acknowledged in public print. Based on conversations and correspondence with laboratory personnel, I would guess that only about one half of all laboratory-acquired disease has been acknowledged by publication.

The extent and quality of medical attention available without cost to laboratory personnel engaged in the study of infectious disease is an important element in recognition of laboratory-acquired illness. Our policy at Detrick is that every illness in an occupationally exposed person is considered to be an occupational illness until medically proved to be otherwise. By law, occupational illness is entitled to free medical care. A medical staff is maintained to provide the necessary examinations without charge to the employee, and without loss of pay, and without loss of accrued vacation-time or of accrued sick leave. In other words, the employee is carried on a "duty" status during medical examination or treatment; this is justified from the government's point of view because his illness provides valuable medical data to our physicians. In many instances, by routine serological examinations, we find evidence of infection in the absence of clinical illness. As a result of such careful medical attention to our employees, many illnesses and infections are found which would otherwise escape notice, or would be passed off as a severe cold or mild attack of influenza.

* Read before the First North American Conference of Medical Laboratory Technologist, Quebec, Canada, June 1956. Published in the September 1956 issue of the Canadian Journal of Laboratory Technology.

Accidents

Among the known accidents which cause infection, the most common is probably aspiration of infectious fluid through a pipette. Use of pipetting devices has been mandatory in our laboratories for several years, and so the pipette is now a rare source of infection. However, the second most generally common type of accident continues to give us trouble, namely, that connected with use of a syringe. All of you are familiar with these—such as, loose needle, broken tips, broken barrel, and self-injection. Mandatory use of a locking metal tip is helpful, but so far we have found no way of preventing accidental self-injection. The infectious hazards accompanying known accidents, and associated recommendations, in connection with the use of the pipette, hypodermic syringe, centrifuge, and inoculating needle, have all been published recently in your own professional journal[2(a-d)] and I refer you to these articles for further details.

Unknown Sources of Infection

In considering the unrecognized sources of laboratory infection, it must be assumed that every laboratory manipulation with a culture lets loose some organisms into the air. Fortunately for all of us who have worked, or do work, in microbiological laboratories, most of the organisms we study have a low invasiveness for man, and do not penetrate the visibly unbroken skin. In any event, whether or not the technician becomes infected depends upon the dosage he receives, virulence of the organism, route of infection, and resistance of the individual.

Most common laboratory procedures liberate only a few organisms. Most of us absorb these without harm, except for some organism like *Pasteurella tularensis*. A small dosage of this organism, acquired during ordinary operations, is probably enough to infect, and is the reason why most laboratory persons working with tularemia sooner or later acquire the disease.

The most important idea I wish to leave with you concerning the unrecognized sources of infection is that they originate within a few inches of the worker's nose.

Determination of Risk

The very practical question then arises as to how one can determine the risk to himself in connection with a specific technique and a specific organism.

The best way is by use of an air sampler, either a sieve sampler using an agar plate, or a liquid impinger. These have been described elsewhere.[2a,3] The one hour slit sampler[4] is particularly valuable when bacterial concentrations in the air are low and the technique under examination requires more than 5 minutes. Unfortunately these samplers are not commercially available, but a good machinist, or glass blower

in case of the liquid impinger, can make them. During laboratory procedures with organisms such as *Pasteurella tularensis* and *Brucella melitensis,* air sampling is apt to yield no organisms, but with vegetative cells with an environmental stability equal to that of the non-pathogenic *Serratia marcescens,* the organisms are easily recovered.

One should not underestimate the value of cotton swabs to sample surfaces of floors, table tops, test tubes, and fingers. It may be assumed, when surface sampling shows infectious organisms, that some of these find their way to the skin, mouth, and nostrils, and when the particles are under 5 to 10 microns in diameter, into the lung.[5]

Settling plates have some value but their collection efficiency is lower than that of other collecting devices. Studies performed at the National Institute of Health show that, on an average, open plates collect only about one tenth the number of bacterial particles collected by sieve samplers.[6] Also the open plate, as a sampler, tends to collect larger particles which fall rapidly to surfaces rather than the small particles which remain suspended for some time in the air. Nevertheless there is ample justification for the use of open plates as a non-quantitative sampler. Laboratory technicians should be encouraged to expose open petri plates containing suitable culture media when questionable operations are being carried out. A better procedure would be to perform the proposed operation using a relatively harmless "test organism" such as *Serratia narcescens.*

In animal rooms, a convincing method of sampling which deserves more use is that of placing a control animal or animals in the same cage with, or adjoining to, the experimentally infected animal. This procedure not only gives some estimate of the risk to the experimentor, but also provides a check on the validity of the experiment, in case animals with different sized inocula are in the same or adjoining cages. If a cage mate control becomes infected by association, then some thought should be given to the effect of the supplementary accidental dosage upon the experimentally inoculated animals. Experiments show that there may be significant cross infection,[7] and in some reported experiments with *Bacillus anthracis* allowance has been made for this.[8]

One should not necessarily assume that there is no risk to man, if air sampling is negative or if control animals remain uninfected. We have had instances of infection of man during operations with *P. tularensis,* in the absence of positive air samples. Likewise, there may be cases in which man is more susceptible than an animal. A recent report from our laboratories showed illness was caused in man by the subcutaneous injection of a vaccine containing an amount of living Venezuelan

equine encephalomyelitis virus which was too small to infect a very large number of several kinds of laboratory animals.[9]

Prevention of Infection

As for means of preventing laboratory-acquired illness, the single most useful piece of equipment is the so called bacteriological safety cabinet.[10] The cabinet may be used with or without attached gloves or port hole panels. The principles involved in the use of these cabinets are the same as those employed by chemists working with toxic gases and physicists handling radioisotopes, namely, (1) the interposition of glass between the worker's face and the operation and (2) the maintenance of a flow of air into the cabinet. These cabinets may be simple, or very complex. As long as the spatial requirements regarding angle of the glass and distance between the cabinet floor and beginning of the inclined glass are observed, the cabinet may be constructed in several sizes, and with several different materials. Many laboratories will find that a plywood cabinet is sufficient if it is lined on the working surface with galvanized metal or stainless steel and if the wood is painted with chemically resistant paint.

Sterilization of exhaust air from the cabinet is always a problem. The method selected will depend upon the amount of infectious aerosol in the cabinet and the size of the cabinet. For all ordinary laboratory operations conducted in the cabinet, filtration of the air through spun glass fiber filter pads will be adequate. These filters have bacterial filtering efficiencies of 98 per cent or better.[11] The size of the entrance for the arms is important because of the need for an air flow of no less than 50 linear feet a minute. For instance, a cabinet with a 72″ x 10″ (5 sq. ft.) opening would need an exhaust fan with a capacity of 250 cu. ft. a minute. Some excess capacity is desirable, so in this instance a 300 c.f.m. fan is the one of choice. There is an electric grid incinerator commercially available but it has a capacity of only 100 c.f.m. Incinerators using Bunsen burners will handle only about 10 to 15 c.f.m. A 300 c.f.m. filter can be purchased for about $300, but there is no reason a simpler model cannot be made locally, by omitting the electrical self-sterilizing unit. The filter material itself costs only 15 to 50 cents a square foot.

Summary

Laboratory-acquired illness is the result of the inter-relationships of the operator, the operation, the organism, and the equipment. A significant change in any one of these factors can result in infection. Good technique and good housekeeping can go a long way in preventing infections, but some procedures cannot be made safe without special equipment. However, equipment

is no substitute for safe technique which is based upon active participation by the worker at the laboratory bench in the process of establishing safe practices.

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SPINAL FLUID SEDIMENTATION RATE AS AN AID IN DIFFERENTIAL DIAGNOSIS OF ORGANIC REACTION VERSUS FUNCTIONAL REACTION*

Preliminary Report

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Purpose

Differential diagnosis of organic reactions from functional reactions can be a difficult problem for the diagnostician. Organic reactions may simulate the functional reaction and vice versa, or they may exist at the same time.

Erythrocyte sedimentation rates have been used as an indicator of systemic organic reactions with some success.[1]

It was decided on an empirical basis to use mixtures of patients oxalated blood and patient's oxalated cerebrospinal fluid to note if differences in sedimentation rate occurred in organic and/or functional reactions. As a standard normal saline was mixed with the patients' oxalated blood.

No attempt has been made to determine the biochemical or biophysical reasons for the results.

Methods and Procedures

The following tests and special sedimentation procedures on the blood and/or cerebrospinal fluid for two hundred and thirty-two of the cases were carried out:

1. Cerebrospinal fluid cell count.
2. Total protein on spinal fluid using the Turbidimetric Method of Denis and Ayer, modified to our Cendo-Sheard-Sanford Photelometer.
3. Packed red cell volume Wintrobe method.
4. Special Sedimentation rate.

A. Equipment for special sedimentation rate.

1. Westergren tubes.
2. Rack for Westergren tubes.
3. Shell vials with dried double oxalate.
4. 5 or 10 ml. test tubes.

B. Reagents.

1. Sodium Chloride—0.9% solution.
2. Anticoagulant Solution:
   Double oxalate made up of:
   1. Ammonium oxalate—12 gms.
   2. Potassium oxalate—8 gms.
   3. Distilled water to make 1 liter.
   4. Pipette 0.5 ml. of above double oxalate solution into shell vials and evaporate to dryness in oven (not over 70° C.) This amount prevents 5 ml. of blood from clotting. These shell vials are used to oxalate patient's blood and C.S.F.

C. Technique of Special Sedimentations.

1. Collect 5 ml. of patient's blood in shell vial containing double oxalate as noted above and mix well.

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2. Place 5 ml. of C.S.F. in similar oxalated shell vial and mix well.
3. Fill 3 Westergren tubes to 0 graduation as follows:
   a. Tube I—Patient's oxalated blood.
   b. Tube II—A mixture of 1 ml. of patient's oxalated blood and 1 ml. of patient's oxalated C.S.F.
   c. Tube III—A mixture of 1 ml. of patient's blood and 1 ml. of 0.9% Sodium Chloride.

D. Recording Results.

The fall of the erythrocytes in all three tubes is carefully noted and recorded every 15 minutes for a period of 2 hours.

Because of the fact that the blood-cerebrospinal fluid mixture of over 50% cerebrospinal fluid may clot, it was found necessary to employ an anticoagulant for the spinal fluid before the test could be carried out. As a result, 5 ml. of C.S.F. was added to a vial containing dried double oxalate and mixed well to remove the ionic calcium from the cerebrospinal fluid.

In order not to introduce any other diluent to the blood than the spinal fluid or physiological saline, the dried double oxalate was again used.

Normal values were those of Cecil and Dejong.[3, 4]

Results

For the purpose of economy of space the tables have been condensed from the total of 232 cases studied. These patients were given the following examination: physical, neurological, psychiatric and psychologic tests.

Table I.

This table is a selection of 10 cases with pure functional illness. The time limit for the sedimentation rate was 60 minutes and 120 minutes. The findings are within the normal limits. It is to be noted that there is no gross difference of more than 1 mm in the sedimentation rate in mixtures of cerebrospinal fluid or

TABLE I

Normal Sedimentation Rate in Pure Functional Illness

Case No.	Diagnosis	Time	Blood Sed. Rate	Blood + CSF 1:2	Blood + Saline 1:2	Difference Saline vs. CSF	CSF Protein	CSF Cells	Blood Vol. %	CSF Pressure
25	Involutional reaction	60	4.5	2	2	0	21	1L	49	64
	Mixed Type	120	15	5	4	—1				
32	Schizophrenic reaction	60	1.5	1.5	1.5	0	16	0	47	120
	Undifferentiated	120	3	3.5	4	0				
55	Paranoid condition	60	4.5	2.5	2.5	0	24	0	46	112
		120	11	5	4	—1				
193	Schizophrenic reaction	60	9	2	2	0	25	2L	38	120
	Paranoid, Type Chronic	120	28	6	5	—1				
196	Schizophrenic reaction	60	18	3	3	0	25	1L	45	180
	Pseudoneurotic type	120	43	8	7	—1				
188	Involutional reaction	60	25	3	3	0	12	0	42	140
	Mixed type	120	52	8	6	—2				
132	Compulsive obsessive	60	18	4	4	0	20	0	41	140
	reaction	120	35	8	8	0				
133	Involutional reaction	60	9	3	3	0	22	2L	45	128
	Paranoid, chronic	120	25	5	5	0				
70	Schizophrenic reaction	60	11	3	3	0	22	3L	40	120
	Paranoid type, early	120	33	8	7	—1				
98	Schizophrenic reaction	60	1.5	1.5	1.5	0	36	1L	51	148
	Paranoid type	120	4	4	4	0				

of saline. Qualitative and quanititative analysis of the cerebrospinal fluid was also within normal limits.

Table II.

This table is a selection of 10 cases whose initial test results were compared with subsequent tests results in which known organic reactions had supervened. It is to be noted that direct traumatic organic reactions to the central nervous system tended to produce positive differences in sedimentation rate saline vs cerebrospinal fluid.

TABLE II

Comparison of Initial Examination and Subsequent Examination

Case No.	Diagnosis	Time	Blood Sed. Rate	Blood + CSF 1:2	Blood + Saline 1:2	Difference Saline vs. CSF	CSF Protein	CSF Cells	Blood Vol. %	CSF Pressure
47	Schizophrenic reaction	60	11	2	2	0	27	3L	52.5	140
	Multiple Sclerosis in remission	120	32	5	4.5	0				
	17 days later following	60	4.5	1	3.5	+2.5	29	5L		150
	acute Exacerbation of M.S.	120	20	5	6	+1		1P		
106	Schizo-Affective	60	13	3.5	3	0	38	2L	51.5	200
	Reaction	120	42	10	10	0				
	60 days later	60	28	4	5	+1	26	9L	44	210
	Insulin coma reaction	120	51	12	11	−1				
	Thrombosis, cerebral									
	6 months later	60	8	3	3	0	22	2L	47	120
		120	23	7	6	−1				
167	Schizophrenic reaction	60	99	28	23	−5	13	1L	38	140
	Paranoid Type	120	106	62	52	−10				
	Pregnancy 5 months									
	Lacerations wrists.									
	6 mos. later attempted	60	96	17	22	+5	49	2P	35	170
	suicide. Multiple fractures, sternum, hands, malar bone. Cerebral	120	117	42	55	+13				
	Contusion 6 days after falling 8 stories									
	11 days later	60	86	13	15	+2	60	1L	36	156
		120	110	29	34	+				
	14 days later	60	65	12	12	0	86	1P	38	164
		120	101	26	26	0				
220	Schizo-affective reaction	60	10	3	3	0	17	0	46	178
	Attempted suicide	120	27	5	5	0				
	Superficial laceration of head									
	Post Operative Trans-	60	30	3	5	+2	36	3L	41	340
	orbital lobotomy	120	62	7	13	+6				
	11th day									
214	Schizophrenic reaction	60	27	3	3	0	38	1L	45	120
	Catatomic type	120	68	7	7	0				
	Post Operative	60	43	5	6	+1	112	74 rbc	41.5	118
	Transorbital lobotomy	120	90	16	16	0				
	10th Day									
226	Involutional reaction	60	6	2	1.5	0	51	1L	46	164
	Arteriosclerotic H.D. mild	120	18	6	4	−2				
	Post Operative	60	21	4	5	+1	65	rbc 5000	43	265
	Transorbital lobotomy	120	48	6	6.5	0		10L		
	5th Day									
	8th Day	60	36	6	7	+1	42	2L	46	198
		120	76	13	15	+2		1P		

TABLE II (Continued)

Case No.	Diagnosis	Time	Blood Sed. Rate	Blood + CSF 1:2	Blood + Saline 1:2	Difference Saline vs. CSF	CSF Protein	CSF Cells	Blood Vol. %	CSF Pressure
136	Schizophrenic reaction	60	1	2	2	0	23	1L	40	
	Paranoid Type, chronic	120	2.5	4	3	—1				
	60 days later after	60	12	4	2.5	—1.5	19	1L	53	
	severe insulin coma reaction	120	49	11	10	—1				
167 Concluded	21 days later	60	35	5	7	+2	69	1L	39	120
		120	75	12	16	+4				
	28 days later	60	30	4	4	0	88	1L	39.5	120
		120	55	11	11	0		1P		
140	Involutional reaction. Addiction, barbiturate	60	15	2	2	0	22	1L	36	190
		120	38	5	5	0				
	Pernicious Anemia									
	6 days later following	60	13	11	4	—7	25	5L	36.5	198
	convulsion	120	74	28	9	—19				
208	Agitated Depression	60	15	3	3	0	28	0—	47	80
	Hypertensive c-v. disease	120	53	8	8	0				
	Post operative transorbital lobotomy 8th day	60	14	13	15	+2	140	6L	42	130
	Cardiac decompensation	120	53	35	35	0		5P		
212	Involutional reaction	60	10	2	2	0	30	0	43.5	120
	Mixed type	120	26	4.5	4.5	0				
	Post Operative	60	35	4	6	+2	79	1233 rve		138
	Transorbital lobotomy	120	57	22	15	—7		20P	39	
	7th Day							15L		
	Post Operative	60	25	3	3	0	160	rbc 800	38	340
	9th Day	120	56	7	7	0				
	Post Operative	60	18	3	3	0	53	rbc 250	42	600
	13th Day	120	43	6	5	—1		ube 27		
	Post Operative	60	26	3	2.5	0	42	6L	38	180
	19th Day	120	51	7	6	—1				

Table III.

This table is a selection of 10 cases with faster sedimentation rates saline vs cerebrospinal fluid. An attempt has been made to arrange them in descending order. From a clinical viewpoint it seemed that the more severe the reaction the greater the positive difference.

TABLE III

Faster Sedimentation Rate Saline vs. Cerebrospinal Fluid

Case No.	Diagnosis	Time	Blood Sed. Rate	Blood + CSF 1:2	Blood + Saline 1:2	Difference Saline vs. CSF	CSF Protein	CSF Cells	Blood Vol. %	CSF Pressure
6	Schizophrenic reaction	60	98	25	35	+10	85	rbc 1600		
	Paranoid type chronic	120	108	55	85	+30		56L	40	380
	Post Operative partial Prefrontal lobotomy 3rd Day							44P		
		60	63	10	21	+11	48	8L	55	180
	9th Day Post Operative	120	95	35	46	+11				
57	Schizophrenic reaction	60	65	10	20	+10	58	25 wbc	46	240
	Pseudoneurotic type chronic. Post Operative partial Prefrontal lobotomy 8th Day	120	70.5	21	45	+24				

TABLE III (Continued)

Faster Sedimentation Rate Saline vs. Cerebrospinal Fluid

Case No.	Diagnosis	Time	Blood Sed. Rate	Blood + CSF 1:2	Blood + Saline 1:2	Difference Saline vs. CSF	CSF Protein	CSF Cells	Blood Vol. %	CSF Pressure
65	Involutional reaction Insulin coma reaction Cerebral Thrombosis	60 120	60 82	11 27	16 35	+5 +8	27	0	47	120
231	Schizophrenic reaction Paranoid type acute Tuberculome—1½ years ago	60 120	54 88	6 16	10 25	+4 +9	16	2L	46	170
163	Lobar pneumonia Tabo paresis	60 120	98 105	38 72	42 82	+4 +10	33	4L	40	150
74	Involutional reaction Hypertensive c-v Disease	60 120	37 77.5	6 13	8.5 19	+2.5 +6	21	2L	47	140
93	Schizophrenic reaction Pseudoneurotic type Severe insulin coma reaction	60 120	35 82	7 15	9 16	+2 +1	31	1P	46	122
95	Carcinoma, metastatic, Laminectomy-chordotomy Anemia	60 120	89.5 106	13 32	14 31	+1 −1	46	4L 3P	44	80
79	Involutional reaction Arachnoiditis Laminectomy-chordotomy Anemia Addition-opiate-barbituate	60 120	74 107	10 24	11 26	+1 +2	38	3L	37	150
33	Hypertensive c-v disease Hypertensive Encephebpathy	60 120	48.5 92.5	8 21	9 22	+1 +	42	2L	43	230

Discussion

Laboratory tests are an aid to the clinician and do not replace careful clinical evaluation. Central nervous system reactions can be very puzzling unless marked involvement occurs or focal objective clinical signs are present. Any assistance to establish the diagnosis is helpful.

As in all human activities error may arise particularly if different technologists perform tests and read results. We noted that less error occurred when one or two technologists were trained in the procedures and observed accurately the time for recording. Correct interpretation of the meniscus is important.

In general it was observed that the higher the erythrocyte sedimentation rate the higher the sedimentation rate in cerebrospinal fluid and saline. Some cases revealed no difference in the sedimentation rate, saline vs cerbrospinal fluid and it was

(Continued on Page 322)

Table IV.

This table is a selection of 10 cases with slower sedimentation rate saline vs cerebrospinal fluid. The arrangement has been in descending order. It would appear that more systemic organic reaction is associated with the negative difference. The more marked the difference the more serious the clinical picture.

TABLE IV

Slower Sedimentation Rate Saline vs. Cerebrospinal Fluid

Case No.	Diagnosis	Time	Blood Sed. Rate	Blood + CSF 1:2	Blood + Saline 1:2	Difference Saline vs. CSF	CSF Protein	CSF Cells	Blood Vol. %	CSF Pressure
31	Cirrhosis of Liver Alcoholism, acute & chronic Emotional immaturity reaction	60 120	79 99.5	16 20	4 10	−6 −10	36	2L	41	140
157	Involutional reaction Paranoid type chronic Sarcoidosis Arteriosclerotic H. Disease Alcoholism, chronic Cystitis, chronis	60 120	100 108	22 51	16 44	−6 −7	16	0	36	94
145	Cerebral Thrombosis Hypertensive c-v Disease Alcoholism, chronic	60 120	88 95	35 84	30 78	−5 −6	34.5	11L	44	80
104	Cirrhosis of Liver Alcoholism, acute & chronic Cerebral hemorrhage	60 120	89 96	8 20	5 11	−3 −9	610	rbc 4800	41	300
144	Miscarriage 4 days prev following 5 mo. preg. Cerebral Emboli	60 120	78 101	12 28	9 23	−3 −5	45	3L	40	160
110	Alcoholism, acute Bromide intoxication (300 mg%) Emotional instability reaction	60 120	72 100	17 40	14 38	−3 −2	31	1L	42	82
215	Schizophrenic reaction Paranoid type Pregnancy 5 months	60 120	43 70	4 7	3 7	−1 0	4	1L	36	140
109	Involutional reaction Paranoid, chronic Malnutrition	60 120	29 62	3 5	2 6	−1 +1	22	1L	46	100
12	Bromide Intoxication (315 mg%) Schizophrenic reaction Undifferentiated	60 120	82 125	15 33	14 31	−1 −2	38	3L	40	60
88	Involutional reaction Paranoid, chronic Hypothyroidism Anemia	60 120	60 90	3 7	2 7	−1 0	51	1L	32	124

DISCUSSION—(Continued from Page 320)

assumed that an equilibrium had been established in the blood-brain barrier.

Some cases showed no gross abnormality in the qualitative and quantitative analysis of the cerebrospinal fluid, but distinct abnormality in the erythrocyte sedimentation rate and spinal fluid sedimentation rate. Similarly, where an equilibrium apparently had been established there was evidence of qualitative and quantitative abnormality in the cerebrospinal fluid analysis. A careful evaluation would therefore prompt caution, until the picture was clear.

Table I was presented to show an average value in pure functional reaction. It illustrates erythrocyte sedimentation rates will be in normal limits and no difference in the spinal sedimentation rates saline vs cerebrospinal fluid for uncomplicated functional reactions.

Table II shows findings on admission compared with subsequent results in known organic reactions. Case #167 illustrated gross organic reaction on admission and serial studies six months later. A negative difference saline vs cerebrospinal fluid was present on admission and following severe trauma of a multiple nature there was a positive difference saline vs cerebrospinal fluid. As recovery took place an equilibrium tended to be established. The clinical findings in the functional reaction were not altered following the trauma. The protein values increasing may have been a reaction of recovery not an expression of increasing pathology. This patient ultimately recovered.

Case #212 revealed positive difference, saline vs cerebrospinal fluid following transorbital lobotomy but then an equilibrium was reached in spite of abnormal findings in the spinal fluid analysis. From a clinical viewpoint this patient did not show gross signs of serious neurological disturbance. The spinal fluid pressure of 600 mm was a complete surprise to those working with this patient. Recovery was uneventful.

Table III illustrates the findings in which positive difference occurred in sedimentation rate saline vs cerebrospinal fluid. The indications seem to bear out the fact that acute organic reaction in the central nervous system is associated with a positive difference.

Table IV illustrates negative differences in the sedimentation rate saline vs cerebrospinal fluid. The cases presented are those involving more diffuse systemic reactions with secondary involvement in the central nervous system.

Before absolute criteria can be established more diversified cases should be studied particularly of a neurologic nature. It seems to us that the relationship is a dynamic one, organic central nervous system reactions can produce change in systemic

reaction and the reverse is true. When the clinical picture is that of combined functional and organic reaction the laboratory tests aid in weighing the importance of each and can be of help in timing therapy of each. The clinician can be alerted by utilizing the spinal fluid sedimentation test in his evaluation of organic reactions and/or functional reactions.

Conclusions

1) A new test (spinal fluid sedimentation test) is presented to aid the clinician in diagnosis of 1) systemic organic reaction involving the central nervous system; 2) organic reaction in the central nervous system with secondary systemic reaction and 3) purely functional reaction.

2) The more severe the organic reaction in the central nervous system the greater the difference in the spinal fluid sedimentation rate either positive or negative.

3) It is suggested that the more positive the difference, saline vs cerebrospinal fluid the more acute the reaction in the central nervous system. The more negative the difference, saline vs cerebrospinal fluid, the more systemic the organic reaction.

4) Normal findings in sedimentation rate of blood, saline vs cerebrospinal fluid and qualitative and quantitative examination of cerebrospinal fluid indicate uncomplicated functional reactions.

5) Repeated spinal fluid sedimentation rates can be used as a measurement of equilibrium being established in combined organic and functional reactions.

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ASMT SILVER ANNIVERSARY CONVENTION

Palmer House, Chicago, Illinois

June, 1957

Theme: GROWTH THROUGH SERVICE

Another peak in the history of our society is just over the ridge of 1956. And it is a silver peak gleaming in the light of the service of all its members and friends throughout the years. Some have served and climbed to the peak from the "sea level," others have joined us at various levels and some will join us for the first time at our 25th anniversary.

Papers: The Program Committee invites you to write and to present papers at this convention. The number and the subject matter of qualified papers submitted will once again determine the type and the arrangement of the program.

Awards: Papers may be submitted for the many awards. These awards and the rules for writing the papers will be listed and sent to members in October. **Read** and **follow** the rules as directed, otherwise, your paper will not be eligible.

Deadline: Deadline for submitting the **title** of your paper to the Program Committee is December 1, 1956. The finished paper need not be submitted until March.

Guest Speakers: The final invitations have not been sent out as yet, but a glimpse through the keyhole may show you

1) a noted professor of medicine who has recently returned from two trips to India;
2) a group of specialists in blood banking techniques;
3) a professor in a department of animal pathology;
4) a doctor who is renowned for his lectures and beautiful slides in hematology.

Symposiums: Yes, there may be—you can't tell!

Conferences: Time will be allowed for conferences on legislation, membership, recruitment, public relations, publications, possibly laboratory supervision and education.

Refreshments and Workshops: Plans are being made for several of these including mycology, cytology, hematology, bacteriology, possible micro chemistry techniques and medical photography.

Medical photography is a new type of workshop for us. Many medical technologists, especially those in smaller hospitals are doing this, but most of us have not had time to take training in medical photography. We can have the assistance of some excellent medical photographers, **but** they need to know how they can help most in the two or three hours allotted. What are your problems—lighting techniques, copy work, high-lighting skin eruptions, color photography or just plain focusing? Send your problems on photography only, to Mrs. Selma N. Earle, M.T. (ASCP), Laboratory Supervisor, Mt. Sinai Hospital, Chicago 8, Illinois.

Remember:

1. List of awards and rules for writing papers will be sent to you in October.
2. Submit **titles** to papers by December 1, 1956. The finished paper may be sent any time after reading the rules up to the deadline of March, 1957 to

Mrs. Doris S. Whitney, M.T. (ASCP)
Program Chairman, 1957 ASMT Convention
65 West Irving Park Road
Roselle, Illinois

ARTICLE IV
Membership

Section 1. The membership of this Society shall consist of the following classes:

(a) Active Member. Any person who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in microbiology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a certificate in chemistry or microbiology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, and is in good standing with that Registry, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more semester credit hours from an accredited college of which at least twenty-seven hours are in science including chemistry and/or biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.

(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing or special committee of this Society.

(c) Student Member. Any person enrolled in an approved School of Medical Technology is eligible for Student Membership in this Society. He may enjoy this membership until he becomes a registered M.T. (ASCP) by the Board of Registry of Medical Technologists of ASCP or until he becomes ineligible to take the examination for such registration. Upon successful completion of the Registry examination he shall automatically be billed for Active Membership in this Society. A student member is entitled to all the privileges of membership in this Society except voting, holding office, and serving on any Board or standing or special committee of this Society.

(d) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendation for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording

* Remove pages 325, 327, and 329 and paste in the proper places in your copy of the By-laws. Reprints are available upon request.

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AMONG THE NEW BOOKS

TECHNICAL METHODS AND PROCEDURES of the American Association of Blood Banks. Revised Edition 1956. Burgess Publishing Company, Minneapolis, Minnesota. 111 pages. Tables. $3.00.

For practical purposes a copy of this should be in every blood bank laboratory. Compiled by an editorial committee familiar with each step, the techniques are those found in many other books, but are here condensed for easy reference.

Chapters range from the reception of donors, through registration to their post phlebotomy care, thence to the processing of blood and details of techniques concerned. Record keeping and storage are also included. The rest of the chapters and the appendix are devoted to the laboratory techniques involved in blood banking procedures.

THE MORPHOLOGY OF HUMAN BLOOD CELLS: L. W. Diggs, M.A., M.D., Professor of Medicine and Director of Medical Laboratories, University of Tennessee and City of Memphis Hospitals, Consultant in Hematology, Armed Forces Institute of Pathology, Washington, D. C., Dorothy Sturm, Instructor, Memphis Academy of Arts, Ann Bell, B.A., Instructor in Medicine, University of Tennessee. W. B. Saunders Company, Philadelphia and London, 1956. 181 pages. 54 figures. Color Plates I to XXXI. $12.00.

This volume is primarily for the student technologist and for the medical technologist who examines blood smears daily in the hospital, clinic, or physician's office laboratory. As noted in the preface, "emphasis is placed on the characteristics of individual cells and on differential morphology rather than on diseases of the blood and blood-forming organs." Miss Sturm has done a splendid piece of work in her water color reproductions of the cells. The terminology is that recommended by the Committee for Clarification of Nomenclature sponsored by the ASCP several years ago.

Nearly half the book is devoted to Chapter I on the Normal Blood Cells and Their Progenitors. Other chapters include the Fixed Tissue Cells of the Bone Marrow, Normal and Abnormal Mitoses, Abnormalities in the Morphology of Erythrocytes, Pathologic Leukocytes, and Pathologic Megakaryocytes and Thrombocytes. A ten-page chapter on the L.E. Cell is generously illustrated. Finally there is another brief chapter on techniques. Altogether, this would be another useful addition for the teaching laboratory especially.

BLOOD GROUP SUBSTANCES: Their Chemistry and Immunochemistry. Elvin A. Kabat, Departments of Microbiology and Neurology, College of Physicians and Surgeons, Columbia University, and Neurological Institute, Presbyterian Hospital, New York. Academic Press, Inc., New York, 1956. 330 pages. Charts. $8.00.

Valuable in the hospital laboratory library for background material, with a review of the human blood group factors and the methods used in testing for antibodies and antigens, this book is primarily devoted to the blood group substances, their chemical composition and immunochemical characteristics. The chapters on biological effects and chemical structure will serve as an introduction to further study.

MEDICAL RESEARCH: A Midcentury Survey. Volume I. American Medical Research: in Principle and Practice. 765 pp. Volume II. Unsolved Clinical Problems in Biological Perspective. 740 pp. Edited by Esther Everett Lape and Associates for The American Foundation. Boston, Little, Brown & Company. $15.00 the set.

These two volumes contain a wealth of information of interest not only to the scientist, but to the layman as well. The first is devoted primarily to some of the general aspects of basic research—the relationship of bio-

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secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing or special committee of this Society. He shall be exempt from payment of annual dues.

(e) Corresponding Member. Any person who is resident of, and employed as a medical technologist in, a foreign country, who is in good ethical standing, and who is a member of a government—and/or medical association—recognized association of medical technologists in that country, shall be eligible for Corresponding Membership in this Society. He shall make application through the association of which he is a member. A corresponding member shall enjoy all the privileges of this Society except voting, holding office, serving on any Board or standing or special committee of this Society or any of its activities from which he might be excluded by laws governing aliens. A corresponding member shall forfeit all privileges of membership upon changing his place of residence or employment to a geographical area where there is a constituent society of this Society.

ARTICLE VII
Finances

Section 1. The annual dues for active and affiliate membership in this Society shall be eight dollars ($8.00) per annum.

Section 2. The dues for student members shall be on the basis of twenty-five cents ($.25) a month, until such time as he shall be eligible for active membership in this Society. Dues are to be paid as specified on the application form. The full amount due at that time shall accompany the application for membership, and shall be sent to the Executive Secretary of this Society. He may renew his student membership so long as he is eligible for registration.

Section 3. The dues for corresponding members membership in this society shall be four dollars ($4.00) per annum.

Section 4. Dues to the Society shall be payable to the treasurer of the constituent society of the geographical area in which the member is employed or resides, or, in the event no such constituent society exists, to the executive secretary of this Society. Dues are payable on or before July 1 of each fiscal year.

Section 5. (a) Any active, affiliate, or corresponding member who cannot pay dues for the current fiscal year shall notify this Society by sending written notification direct to the executive secretary not later than September 1st of that year.

(b) Any active, affiliate, or corresponding member who fails to pay annual dues to this Society by September 1st shall have his name removed from the membership list.

(c) A person whose name has been removed from the membership list of this Society may be reinstated at any time provided he meets the requirements for membership of both his constituent society and this Society. Unless a person has previously resigned, to be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current year's dues to this Society plus a reinstatement fee of two dollars to this Society.

(d) Any active or affiliate member who changes his residence or place of employment to another state, territory or nation shall become a member of the constituent society of his new residence or place of employment for the remainder of the fiscal year without further payment of dues.

Section 6. A new applicant elected to active, affiliate, or corresponding membership in this Society after January 1st and prior to May 1st shall

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logical, chemical, physical, and mathematical sciences, and their contributions to medicine and to medical research. Of considerable interest to the medical technologist would be the section on research institutions, namely the universities, medical schools and foundations, these last, it is pointed out, more often will promote and finance, rather than maintain the operating laboratories. There is quite a lengthy discussion regarding grants, their various aspects. Another interesting section relates to biological research and how it has been of value in medicine; to agricultural research and its place in microbiology.

The chapter on medical research in hospitals other than teaching hospitals gives a report which shows how large a contribution developed from this source, as well as through volunteer health societies, government and industry.

The second volume discusses more specific medical problems with their current status. Not only the section on virus diseases, but those on cancer and tuberculosis, will be well worth reading.

Other Books Received:

LECTURES ON THE SCIENTIFIC BASIS OF MEDICINE: Volume 4, 1954-55. University of London. The Athlone Press, 1956. Distributed in the U. S. A. by John de Graff, Inc., New York. 21 lectures. 397 pp. Illustrated. Color. $6.50.

ATLAS OF EXFOLIATIVE CYTOLOGY. Supplement 1. George N. Papanicolaou, M.D., Ph.D. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass. 1956. 17 color plates. Text, 44 pp. $4.00.

THERAPEUTIC USE OF ARTIFICIAL RADIOISOTOPES. Edited by Paul F. Hahn, Ph.D., Cancer Research Laboratories, Nashville, Tenn. John Wiley & Sons, Inc. New York. 1956. 414 pp. 19 lectures. Index. Illustrations. Charts. $10.00.

Soranus' GYNECOLOGY. Translated by Owsei Temkin. Publication of the Institute of the History of Medicine, Johns Hopkins University Press, Baltimore. 1956. 258 pp. 2 illustrations. $5.00.

Clinical Recognition and Management of DISTURBANCES OF FLUID BALANCE. John H. Bland, M.D., Associate Professor of Medicine, University of Vermont College of Medicine. 2nd Ed. 1956. W. B. Saunders Company, Philadelphia. 522 pp. 119 figures. 54 charts. $11.50.

FERTILIZATION. Lord Rothschild, G.M., Sc.D., F.R.S. 1956. Methuen & Co., Ltd., London. John Wiley & Sons, New York. 179 pp. 30 figures. 22 tables. Plates V. $3.50.

MEDICINAL CHEMISTRY. Volumes II and III. A Series of reviews prepared under the auspices of the Division of Medicinal Chemistry of the American Chemical Society. 1956. John Wiley & Sons, Inc., New York. Vol. II. 311 pp. Tables. Vol. III. 346 pp. Tables. $20.50 both volumes.

A DICTIONARY OF DIETETICS. Rhoda Ellis, Ph.D., Instructor of Foods and Nutrition, Department of Home Economics, Brooklyn College, N. Y. 1956. Philosophical Library, New York, N. Y. 152 pp. $6.00.

MANAGEMENT OF ADDICTIONS. Edward Podolsky, M.D., Department of Psychiatry, Kings County Hospital, Brooklyn, N. Y., 1956. Philosophical Library, New York. 413 pp. $7.50.

THE OFFICE ASSISTANT in Medical and Dental Practice. Portia M. Frederick, Instructor Medical Office Assisting, Long Beach City College. Carol Towner, Executive Assistant, Department of Public Relations, American Medical Association. 1956. W. B. Saunders Company, Philadelphia. 351 pp. 55 figures. $4.75.

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pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 7. An applicant elected to active, affiliate, or corresponding membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

ARTICLE XIX
Official Publication

Section 2. The official publication shall be made available to all active, affiliate, corresponding and student members of this Society who have paid their dues and are in good standing, and to all honorary members.

N.B. These amendments should be substituted in their proper places in your copy of the By-laws.

ABSTRACTS

TUBELESS GASTRIC ANALYSIS; EVALUATION OF A TECHNIC USING A DYE-RESIN COMPOUND

Maurice L. Sievers and Ralph V. Gieselman. (Veterans Administration Hospital St. Louis, Mo.) Amer. Jour. of Digestive Diseases, N.S. 1, 241-9 (1956).

Consecutive admissions to a general medical ward were subjected to separate tubeless gastric analysis, using the azure A- cation exchange compound and the quininium material, Diagnex. Using the dye-resin compound, the results are obtained by a simple visual comparison colorimetric test on urine without the use of expensive equipment. Data was obtained from 93 patients. It was noted that in 1 patient, severe jaundice and the presence of bile in the urine prevented an interpretation. Tables are presented showing the comparison of the interpretation of Azure A- and Quinine resin results. The results obtained with the new azure A-resin was shown to reproduce the results obtained with the quinine compound, Diagnex. The technic with the azure A-resin is simpler than with the quinine substance and has a comparable accuracy. NMB

INFECTIVITY OF TULAREMIA APPLIED TO INTACT SKIN AND INGESTED IN DRINKING WATER

S. F. Quan, et al. (U. S. Public Health Service, San Francisco, Cal.). Science, 123, 942-3 (1956).

Results of investigation on infectivity of B. tularense by penetration are cited. Such infectivity is studied here quantitatively through the unbroken skin in the albino laboratory mouse and such infectivity is compared with that acquired through the mucosa of the gastrointestinal tract. The average lethal infective dose of B. tularense by penetration through unbroken skin under 10 g. of pressure was 2 to 5 times greater than per os and more than 10^7 times that of intracutaneous inoculation.

Further studies are indicated; difference in penetrability of the skin of mature mice and baby mice; effect of animals in close contact with each other; and the frequent grooming reactions of older mice.

DIRECT-REACTING BILIRUBIN, BILIRUBIN GLUCURONIDE, IN SERUM, BILE AND URINE

Rudi Schmid (U. S. Public Health Service, Bethesda, Md.). Science, 124, 76-7 (1956).

Generally the indirect-reacting form of bilirubin of serum is due to excessive destruction of hemoglobin (example-hemolytic anemia); the direct-reacting occurs when there is intrahepatic or extra-hepatic obstruction to the passage of bile into the intestine. Indirect-reacting pigment is practically insoluble in water below a pH of 8 and direct-reacting bilirubin from serum is water-soluble over a wide range of pH according to recent studies. The instability of the pigment formerly hindering their purification has now been overcome by studying the more stable dipyrromethene diazonium pigments obtained by treating body fluids from jaundiced patients with an

excess of diazotized sulfanilic acid dissolved in dilute hydrochloric acid. This reaction consists of 1 mole of bilirubin reacting with 2 moles of the reagent yielding 2 moles of hydroxypyrromethane diazonium salt.

In one system of purification it was found that direct-reacting bilirubin in the serum of jaundiced patients and almost all of the bilirubin in fresh bile and in the urine of jaundiced patients was of the azo-pigment (B) with an Rf value of 0.25 to 0.30. It was found that the azo-pigment (A) obtained from indirect-reacting serum bilirubin, crystalline bilirubin, and heated bile gave an Rf value of 0.45 to 0.50.

Hydrolysis with 1N hydrochloric acid of the azo-pigment B for 1 hour at 100° C resulted in its conversion to azo-pigment B. This change was also successful with B-glucoronidase of animal or bacterial origin.

Since azo-pigment B was found exclusively in fresh bile and urine from jaundiced patients and the fact that 2 moles of hydroxypyrromethane diazonium salt are produced from 1 mole of bilirubin it would be concluded that direct-reacting bilirubin is conjugated with 2 moles of glucuronic acid. By analogy with other glucuronide formations it may be assumed that the glycosidic linkage occurs at the a, a'-hydroxy group of bilirubin. Where azo-pigment A and azo-pigment B occur together it is suggested that direct-reacting bilirubin may be present as a monoglucuronide, agreeing with an earlier report indicating the separation of two closely related water-soluble fractions of bilirubin from bile. Direct-reacting bilirubin (conjugated bilirubin) predominated in the serum of patients with regurgitation jaundice. Indirect-reacting bilirubin (free bilirubin) was the serum pigment in retention jaundice. In patients with bilirubinuria, the urinary bilirubin was almost exclusively azo-pigment B indicating that the kidneys excrete only conjugated bilirubin.

In conclusion: findings show that in serum, glucuronic acid-conjugated bilirubin gives the direct reaction and that free bilirubin, because of its insolubility in water, requires alcohol to initiate coupling with diazo reagent.

NEW METHOD FOR SHARPENING MICROTOME KNIVES

Olle Hallen, (Univ. of Goteborg, Sweden), Lab. Invest. 5, 308-16, (1956).

A new method for sharpening microtome knives is described. The method discussed is that of lapping against cast iron and glass. A complete discussion of apparatus used is presented along with interferograms of the facets of knives which have been honed, stropped, lapped against cast iron, and lapped against glass. The method of sharpening described has been tested under routine use for six months. It produces an edge that satisfies the highest demands, it is rapid, and it can be learned easily. NMB

BLOOD GROUP FREQUENCIES TABLES

Sidney J. Klein (Univ. of Cal., Los Angeles) Jour. Lab. Clin. Med. 47, 920-2, (1956).

Three tables are presented for potential value as blood-bank desk references. No Claim is made for originality of data. The vast material pertaining to grouping and Rh factor have been resorted and condensed with a view to simplification, comprehensibility, and more ready application. The tables presented in this article are self-explanatory and there isn't any text presented. Table I presents the Rh and Blood Groups. The Rh table gives the Fisher-Race and Weiner phenotype and indicates agglutination results with Rh antiserums. The Blood Group part of table I gives the Fisher-Race genotype and indicates agglutination results with Hr antiserums. Table IA: Rh Subgroup Incompatibility Among D Positive Bloods. This table shows the frequency with which Rh positive persons compatible in respect to the D(Rh_o) factor are incompatible in respect to other Rh factors. Table II: Human Blood Group Systems Frequencies. This table lists the human blood group systems against the clinical importance of antigens and listing the frequencies. NMB

ELECTROPHORETIC DISTRIBUTION OF SERUM GLYCOPROTEINS IN SOME HEMORRHAGIC STATES

Sergio I. Magalini, et al. (Tufts University School of Medicine, Boston), Jour. Lab., Clin. Med. 47, 923-37 (1956).

In this study, 46 individual specimens were studied, distributed as follows: 33 cases of idiopathic thrombocytopenic purpura, 2 cases of thrombotic thrombocytopenic purpura, 5 cases of anaphylactoid purpura, and 4 cases of quinidine induced thrombocytopenic purpura. Electrophoretic migration of serum glycoproteins was studied in 8 healthy persons and 5 patients with "collagen disease." These latter studies were done to check the reliability of the technique used.

Graphs, charts, and photographs accompany this paper explaining in detail the results obtained. The authors show in this paper that typical glycoprotein migration patterns have been found in several bleeding disorders. Combined elevation of albumin and gamma globulin bound carbohydrates seems typical of active idiopathic thrombocytopenic purpura, while isolated elevation in the albumin bound or gamma globulin bound carbohydrates seem typical of drug induced thrombocytopenic purpura and of anaphylactoid purpura. Thus migration of glycoproteins might be of help in distinguishing some varieties of acute anaphylactoid purpura from rheumatic fever, where glycoproteins are found periferentially in the alpha globulin faction. NMB